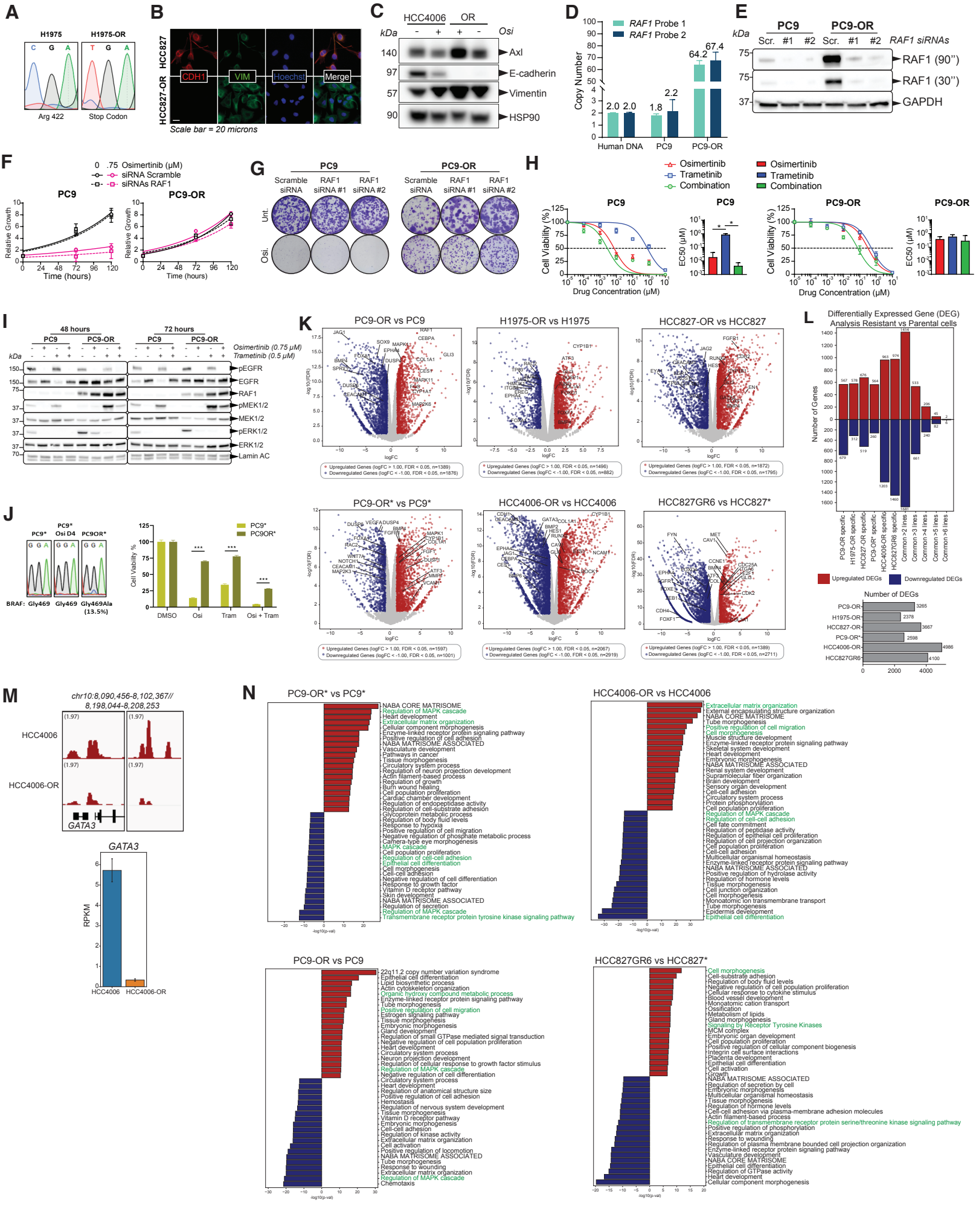
A
H1975
H1975-OR
C G A
T G A
Arg 422
Stop Codon
B
HCC827
HCC827-OR
CDH1
VIM
Hoechst
Merge
Scale bar = 20 microns
C
HCC4006
OR
kDa
Osi
Axl
E-cadherin
Vimentin
HSP90
D
RAF1 Probe 1
RAF1 Probe 2
Copy Number
Human DNA
PC9
PC9-OR
E
PC9
PC9-OR
Scr.
RAF1 siRNAs
RAF1 (90")
RAF1 (30")
GAPDH
F
Osimertinib (μM)
siRNA Scramble
siRNAs RAF1
PC9
PC9-OR
Relative Growth
Time (hours)
G
PC9
PC9-OR
Scramble siRNA
RAF1 siRNA #1
RAF1 siRNA #2
Unt.
Osi.
H
Osimertinib
Trametinib
Combination
Cell Viability (%)
Drug Concentration (μM)
EC50 (μM)
I
48 hours
72 hours
Osimertinib (0.75 μM)
Trametinib (0.5 μM)
pEGFR
EGFR
RAF1
pMEK1/2
MEK1/2
pERK1/2
ERK1/2
Lamin AC
J
PC9*
Osi D4
PC9OR*
BRAF: Gly469
Gly469
Gly469Ala (13.5%)
Cell Viability %
DMSO
Osi
Tram
Osi + Tram
K
PC9-OR vs PC9
H1975-OR vs H1975
HCC827-OR vs HCC827
PC9-OR* vs PC9*
HCC4006-OR vs HCC4006
HCC827GR6 vs HCC827*
logFC
-log10(FDR)
L
Differentially Expressed Gene (DEG) Analysis Resistant vs Parental cells
Number of Genes
Upregulated DEGs
Downregulated DEGs
Number of DEGs
PC9-OR
H1975-OR
HCC827-OR
PC9-OR*
HCC4006-OR
HCC827GR6
M
chr10:8,090,456-8,102,367// 8,198,044-8,208,253
HCC4006
HCC4006-OR
GATA3
RPKM
N
PC9-OR* vs PC9*
HCC4006-OR vs HCC4006
PC9-OR vs PC9
HCC827GR6 vs HCC827*
-log10(p-val)

Figure S1. Genetic and genomic profiles of parental and tyrosine kinase inhibitor- resistant cell line pairs, related to Figure 1. A. Sanger sequencing traces of the mutation identified in H1975-OR cells causing a premature stop codon in the CIC gene. B. Immunofluorescence images of HCC827 and HCC827-OR cells stained with the epithelial marker E-cadherin (CDH1) and the mesenchymal marker Vimentin (VIM). Hoechst staining was used for nuclear DNA. C. Western blot showing upregulation of Axl and downregulation of E-cadherin in HCC4006-OR as compared to parental upon 7 days treatment of 100nM osi, D. Bar graph of RAF1 copy-number in normal human DNA (control), PC9 and PC9-OR cells measured using a TaqMan quantitative PCR assay. E. Western blot showing the levels of for RAF1 in PC9 and PC9-OR cells confirming its overexpression and efficient reduction using siRNAs. Scramble (Scr.); RAF1 (#1 and #2). F. Proliferation curves of PC9 and PC-OR cells 72 hours after siRNA transfection and osimertinib treatment at different concentrations. Data from three independent replicates are shown G. Representative colony formation assays in PC9 and PC9-OR cells after transfection of RAF1 siRNAs and 750 nM osimertinib treatment. H. Dose-response curves of osimertinib, the MEK inhibitor trametinib and the combination of both inhibitors for PC9 and PC9-OR cells (left panels). Bar graph of EC50 values (right panels), n=3. Significance calculated with a paired t test and the Mean ± SEM is shown. I. Western blots showing components of the EGFR/RAS/MAPK signaling pathway in PC9 and PC9-OR cells upon treatment with osimertinib and/or trametinib as indicated. J. Presence of BRAF G469A in PC9-OR* was confirmed by Sanger sequencing but was not observed in the parental PC9* cell line or after 4 days osi treatment (Left). CTG assay demonstrating that while 100 nM Osi + 30 nM Tram nearly eliminates the entire PC9* population, PC9-OR* cells remain significantly more resistant. Data presented as Mean ± SEM with significance calculated using an unpaired t test ***P<0.0005. K. Volcano plots representing differentially expressed genes in PC9-OR, H1975-OR, HCC827-OR, PC9-OR*, HCC4006-OR, and HCC827GR6 cells. Selected top up- and down- regulated genes are labeled. L. (Top) Bar graph of DEGs specifically upregulated or downregulated in each cell line pair as well as across increasing numbers of cell line pairs. (Bottom) Total number of DEGs per cell line pair are represented in the grey bar chart. M. ATAC-seq tracks and gene expression (mean RPKM-/+ 95% confidence interval) at the GATA3 locus. N.Metascape analysis reflecting up and down regulated pathway terms corresponding to DEGs with nearby changes in accessibility in PC9-OR*, HCC4006-OR, HCC827GR6 and PC9-OR cell lines. Selected specific and common terms are highlighted.

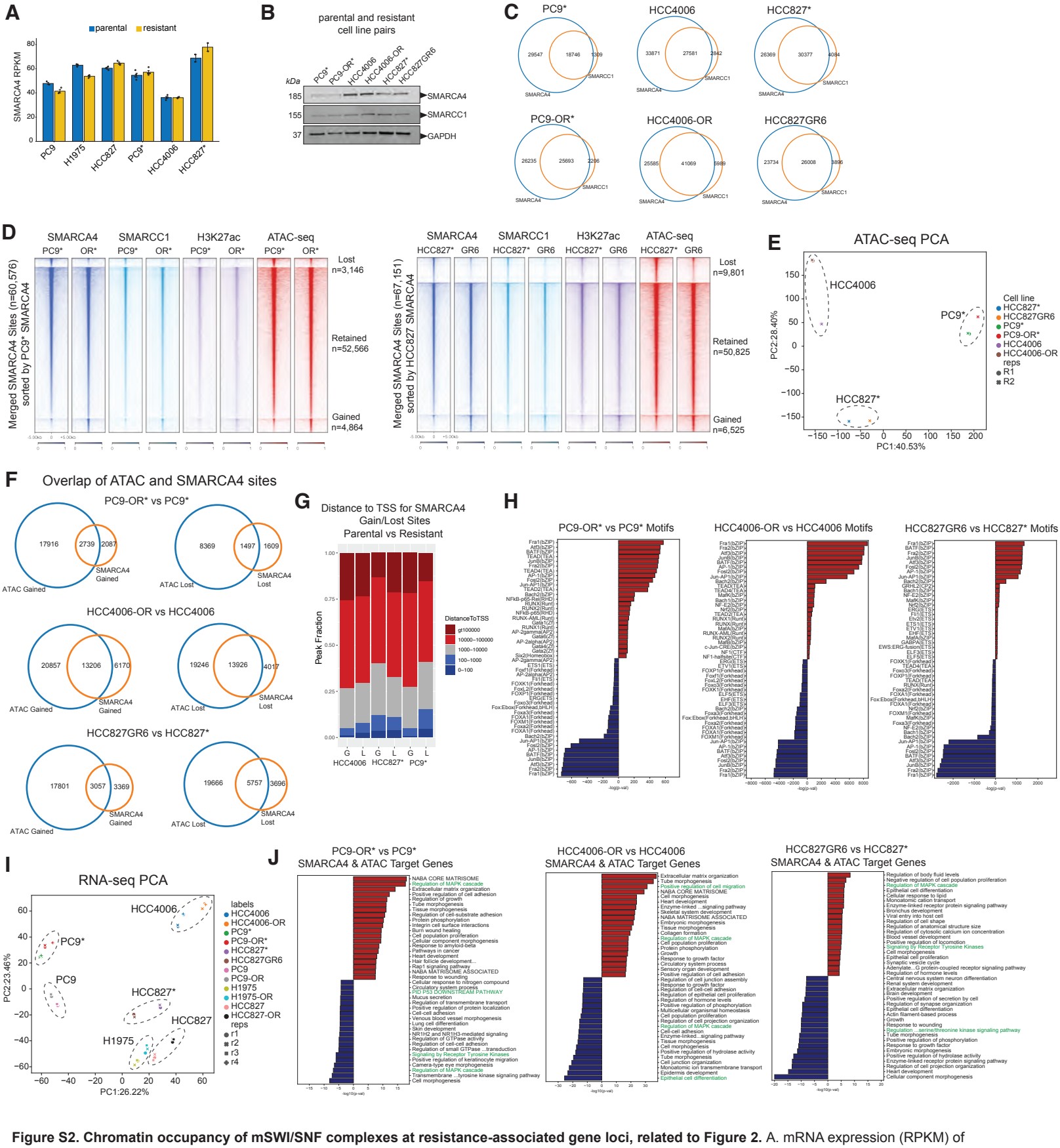

Figure S2. Chromatin occupancy of mSWI/SNF complexes at resistance-associated gene loci, related to Figure 2. A. mRNA expression (RPKM) of SMARCA4 in parental and osimertinib-resistant cell lines. Mean ± SEM is shown. B. Western blot of SMARCA4 and SMARCC1 protein levels in parental and resistant cells. GAPDH is shown as a loading control. C. Venn diagrams showing overlap between SMARCA4 and SMARCC1 peaks (CUT&RUN). D. Heatmap for SMARCA4, SMARCC1, and H3K27ac occupancy levels (CUT&RUN) and ATAC-seq chromatin accessibility in PC9*/PC9-OR* and HCC827*/HCC827GR6 cell lines across merged SMARCA4 sites. E. Principal component analysis (PCA) of ATAC-seq experiments in PC9*/OR, HCC4006/OR, HCC827*/GR6 cell lines. F. Venn diagrams representing overlap between changing (lost and gained) SMARCA4 and ATAC sites in PC9-OR* vs PC9*, HCC827GR6 vs HCC827* and HCC4006-OR vs HCC4006 cells. G. Distance-to-TSS stacked bar graph for SMARCA4 gained (G) and lost (L) sites in PC9*/PC9-OR*, HCC4006/HCC4006-OR and HCC827*/HCC827GR6 cell lines. H. Motifs enriched (red) and lost (blue) under SMARCA4-occupied, accessible sites in the resistance versus parental cell lines. I. Principal component analysis (PCA) of RNA-seq experiments performed across all cell lines. J. Metascape terms for differentially-expressed SMARCA4/accessible target genes for the resistance versus parental state in PC9-OR*, HCC4006-OR, and HCC827GR6 cell line pairs.

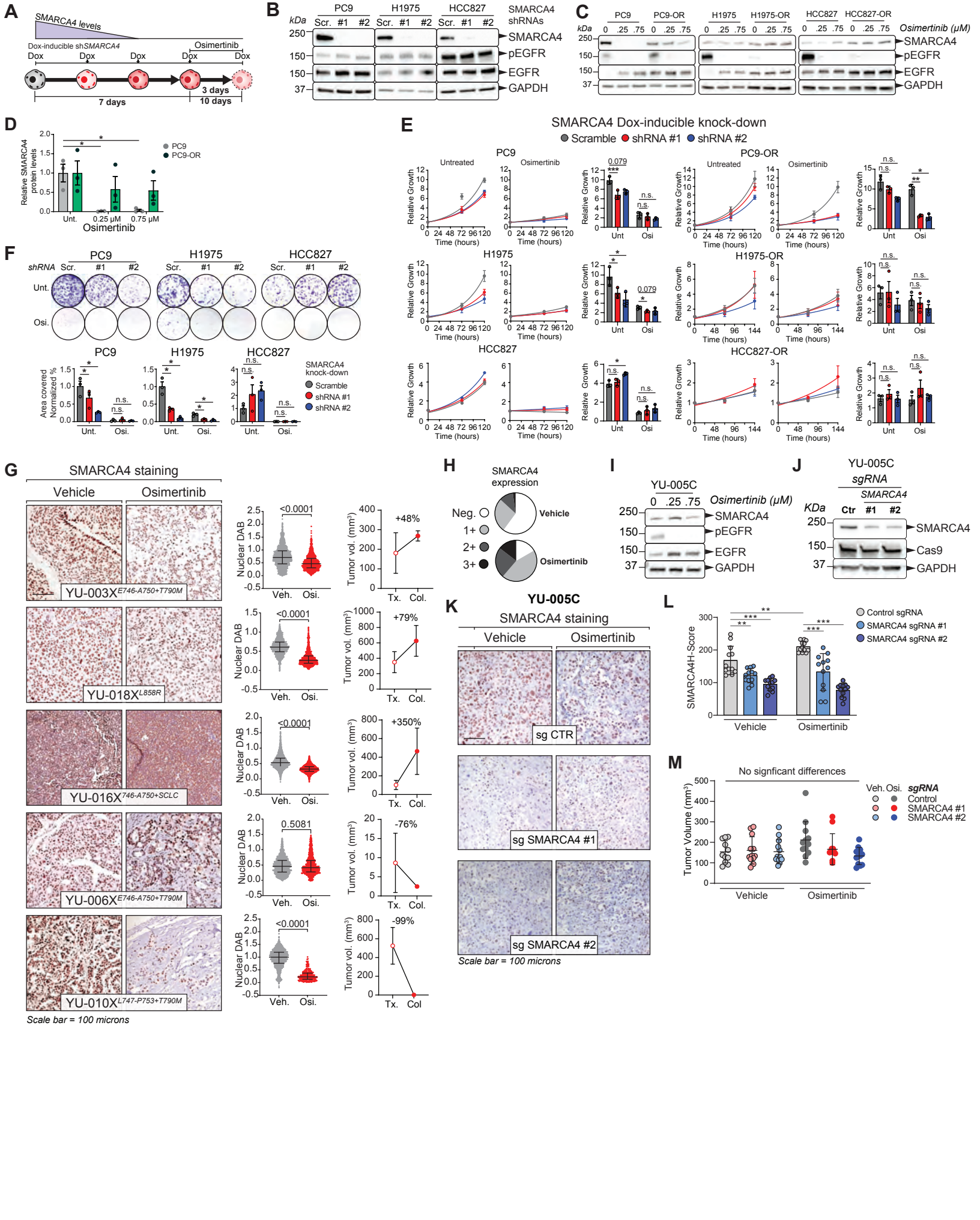
A
SMARCA4 levels
Dox-inducible shSMARCA4
Osimertinib
Dox
Dox
Dox
Dox
Dox
7 days
3 days
10 days
B
PC9
H1975
HCC827
kDa
Scr.
#1
#2
SMARCA4 shRNAs
250
150
150
37
SMARCA4
pEGFR
EGFR
GAPDH
C
PC9
PC9-OR
H1975
H1975-OR
HCC827
HCC827-OR
kDa
0 .25 .75
Osimertinib (μM)
250
150
150
37
SMARCA4
pEGFR
EGFR
GAPDH
D
Relative SMARCA4 protein levels
PC9
PC9-OR
Unt.
0.25 μM
0.75 μM
Osimertinib
E
SMARCA4 Dox-inducible knock-down
Scramble
shRNA #1
shRNA #2
PC9
PC9-OR
H1975
H1975-OR
HCC827
HCC827-OR
Untreated
Osimertinib
Relative Growth
Time (hours)
0.079
n.s.
Unt
Osi
F
PC9
H1975
HCC827
shRNA
Scr.
#1
#2
Unt.
Osi.
Area covered Normalized %
SMARCA4 knock-down
Scramble
shRNA #1
shRNA #2
G
SMARCA4 staining
Vehicle
Osimertinib
YU-003X E746-A750+T790M
YU-018X L858R
YU-016X E746-A750+SCLC
YU-006X E746-A750+T790M
YU-010X L747-P753+T790M
Nuclear DAB
Veh.
Osi.
<0.0001
0.5081
Tumor vol. (mm3)
Tx.
Col.
+48%
+79%
+350%
-76%
-99%
Scale bar = 100 microns
H
SMARCA4 expression
Neg.
1+
2+
3+
Vehicle
Osimertinib
I
YU-005C
Osimertinib (μM)
SMARCA4
pEGFR
EGFR
GAPDH
J
YU-005C
sgRNA
SMARCA4
KDa
Ctr
#1
#2
SMARCA4
Cas9
GAPDH
K
YU-005C
SMARCA4 staining
Vehicle
Osimertinib
sg CTR
sg SMARCA4 #1
sg SMARCA4 #2
Scale bar = 100 microns
L
SMARCA4H-Score
Control sgRNA
SMARCA4 sgRNA #1
SMARCA4 sgRNA #2
Vehicle
Osimertinib
M
No signficant differences
Tumor Volume (mm3)
Veh.
Osi.
sgRNA
Control
SMARCA4 #1
SMARCA4 #2
Vehicle
Osimertinib

Figure S3. SMARCA4 suppression sensitizes Osimertinib-resistant cells and attenuates proliferation in a subset of EGFR-mutant lung cancer cell lines, related to Figure 3. A. Schematic representation of the effects of long-term SMARCA4 inhibition using shRNA lentiviral constructs. After seven days of doxycycline-inducible knock-down SMARCA4 stays knocked-down, and the cells express RFP. B. Western blots of OR cells transduced with shRNAs as indicated. Scramble (Scr.); SMARCA4 (#1 and #2). C. Western blots showing the levels of SMARCA4, phosphorylated EGFR (pEGFR) and total EGFR in PC9 cells, H1975 cells, HCC827 cells and their OR counterparts. D. Densitometry quantification of SMARCA4 western blot in PC9 and PC9-OR cells in the presence of increasing concentrations of osimertinib. E. Proliferation curves of parental and isogenic osimertinib resistant cells one week after shRNA induction and 120 or 144 hours of osimertinib treatment (left panels). Plot of the relative growth of the cells at the proliferation assay end-point (120 or 144 hours). Data from 3 independent replicates are shown (right panels). Osimertinib doses: 750 nM (PC9, PC9-OR, HCC827, HCC827-OR); 1500 nM (H1975, H1975-OR). F. Representative colony formation assay in parental sensitive cells after one-week of SMARCA4 knockdown (top panel). Quantification of the results for independent triplicates is shown (bottom panel).Significance was calculated using a paired t test in E and F. The Mean ± SEM is shown. **P<0.01, *P<0.05. G. IHC staining for SMARCA4 in sections from five PDXs treated either with vehicle or osimertinib (25 mg/kg) (left). Quantification of diaminobenzidine (DAB) intensity (middle) A.U., arbitrary units. Tumor volume change from the start of treatment (Tx.) to the day the tumor was collected (Col.) (right). H. Pie-charts showing the distribution of SMARCA4, phosphorylated EGFR (pEGFR) and total EGFR in cells from a tumor section from YU-005X PDXs treated with vehicle or osimertinib as indicated. I. Western blots of YU-005C cells showing the levels of SMARCA4, phosphorylated EGFR and total EGFR in the presence of increasing concentrations of osimertinib. J. Western blot showing the levels of SMARCA4 and Cas9 in YU-005C cells upon inducible CRISPR/Cas9 knock-down of SMARCA4. Ctr: Control sgRNA; #1: SMARCA4 sgRNA #1; #2 SMARCA4 sgRNA #2. K-L. IHC staining for SMARCA4 in YU-005C tumors upon inducible CRISPR/Cas9 knock-down of SMARCA4. Representative images (K). Bar graph showing the quantification of three microscopy fields each from four different tumors/mice (L). N=12. M. Tumor volume of YU-005C tumors cells injected subcutaneously in mice at the time of treatment initiation. Significance was calculated using a Mann-Whitney test and the Median ± IQR is shown in G, L and M in samples that did not follow a parametric distribution. Significance was calculated using an unpaired t test and the mean ± SEM is shown in samples that followed a parametric distribution in F and G. ***P<0.001, **P<0.01.

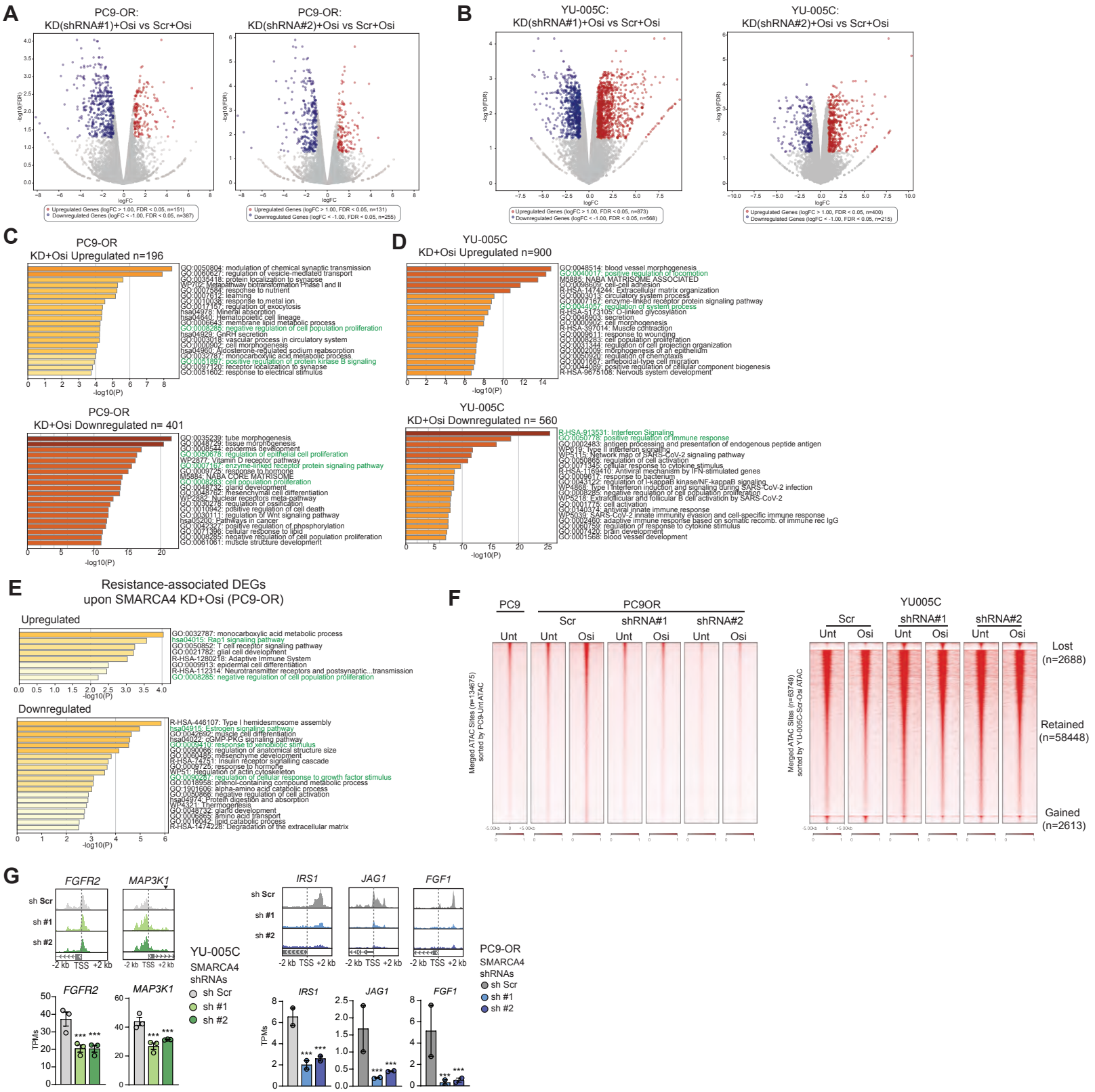

Figure S4. Characterization of chromatin accessibility and gene expression in SMARCA4 knockdown experiments in PC9-OR and YU-005C cells, related to Figure 4. A. Volcano plots reflecting up- and down-regulated genes in SMARCA4 KD shRNA#1-2 compared to shScrambled control condition in Osi-treated PC9-OR cells. Significant genes are highlighted in red (up) and blue (down). B. Volcano plots reflecting significant up- and down-regulated genes in SMARCA4 KD shRNA#1 and #2 compared to scrambled control condition in Osi-treated YU-005C cells. Significant genes are highlighted in red (up) and blue (down). C-D. Metascape analysis reflecting up and down regulated pathway terms corresponding to up- and down-regulated genes in PC9-OR and YU-005C cells upon SMARCA4 KD and osi treatment. The union of genes from both shRNA#1 and #2 were used. E. Metascape analysis performed on up- and down-regulated resistance-associated genes. Key pathways are highlighted. F. Heatmap representation of ATAC-seq experiments performed in PC9-OR and YU-005C cells. G. Differential accessibility and gene expression of selected resistance-associated genes in PC9-OR and YU-005C cells. ATAC-seq peaks at the transcription start site (TSS) ± 2 kb and mRNA expression in transcripts per million (TPM). Mean ± SEM. Significance was calculated using DESeq2. ***P<0.001, *P<0.05.

Figure S5

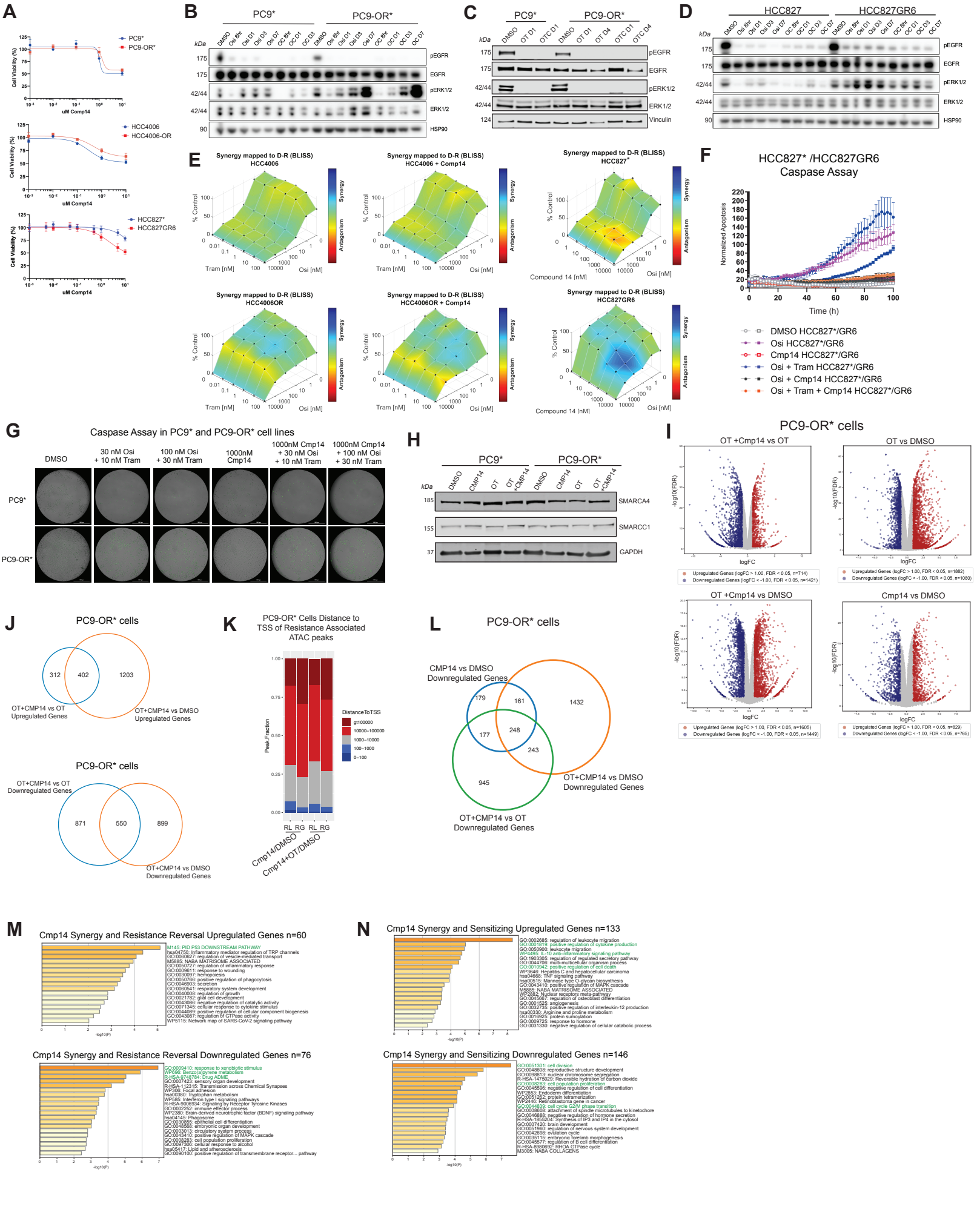
A
PC9*
PC9-OR*
HCC4006
HCC4006-OR
HCC827*
HCC827GR6
Cell Viability (%)
uM Comp14
B
PC9*
PC9-OR*
kDa
pEGFR
EGFR
pERK1/2
ERK1/2
HSP90
C
PC9*
PC9-OR*
Vinculin
D
HCC827
HCC827GR6
E
Synergy mapped to D-R (BLISS)
HCC4006
HCC4006 + Comp14
HCC827*
HCC4006OR
HCC4006OR + Comp14
HCC827GR6
% Control
Tram [nM]
Osi [nM]
Compound 14 [nM]
Synergy
Antagonism
F
HCC827* /HCC827GR6
Caspase Assay
Normalized Apoptosis
Time (h)
DMSO HCC827*/GR6
Osi HCC827*/GR6
Cmp14 HCC827*/GR6
Osi + Tram HCC827*/GR6
Osi + Cmp14 HCC827*/GR6
Osi + Tram + Cmp14 HCC827*/GR6
G
Caspase Assay in PC9* and PC9-OR* cell lines
DMSO
30 nM Osi + 10 nM Tram
100 nM Osi + 30 nM Tram
1000nM Cmp14
1000nM Cmp14 + 30 nM Osi + 10 nM Tram
1000nM Cmp14 + 100 nM Osi + 30 nM Tram
PC9*
PC9-OR*
H
PC9*
PC9-OR*
SMARCA4
SMARCC1
GAPDH
I
PC9-OR* cells
OT +Cmp14 vs OT
OT vs DMSO
OT +Cmp14 vs DMSO
Cmp14 vs DMSO
-log10(FDR)
logFC
J
PC9-OR* cells
312
402
1203
OT+CMP14 vs OT Upregulated Genes
OT+CMP14 vs DMSO Upregulated Genes
PC9-OR* cells
OT+CMP14 vs OT Downregulated Genes
871
550
899
OT+CMP14 vs DMSO Downregulated Genes
K
PC9-OR* Cells Distance to TSS of Resistance Associated ATAC peaks
Peak Fraction
DistanceToTSS
gt100000
10000–100000
1000–10000
100–1000
0–100
RL RG RL RG
Cmp14/DMSO
Cmp14+OT/DMSO
L
PC9-OR* cells
CMP14 vs DMSO Downregulated Genes
179
161
1432
177
248
243
945
OT+CMP14 vs DMSO Downregulated Genes
OT+CMP14 vs OT Downregulated Genes
M
Cmp14 Synergy and Resistance Reversal Upregulated Genes n=60
Cmp14 Synergy and Resistance Reversal Downregulated Genes n=76
N
Cmp14 Synergy and Sensitizing Upregulated Genes n=133
Cmp14 Synergy and Sensitizing Downregulated Genes n=146

Figure S5. SMARCA4/2 ATPase inhibition in Osimertinib- and gefitinib-resistant cell lines reverses chromatin accessibility and gene expression signatures, related to Figure 5. A. Cell viability assays for Cmp14 treatment in PC9*, HCC4006, HCC827* cell lines and their resistant counterparts, n=3 replicates. B. Western blot of pEGFR and pERK1/2 levels across time course treatment of 100 nM Osimertinib (osi) or osimertinib and 1000 nM Cmp14 combo (OC) in PC9* and PC9-OR* cells. Insufficient protein collected for day 7 OC treatment in PC9* cells owing to overt cell toxicity. C. Western blot of pEGFR and pERK1/2 levels across time course treatment of 100nM osimertinib and 30nM Trametinib (OT) or OT and 1000nM Cmp14 combo (OTC) in PC9* and PC9-OR* cells. Insufficient protein collected for day 4 OT and OTC treatment in PC9* cells owing to overt cell toxicity. D. Western blot for pEGFR and pERK1/2 levels across time course treatment of 100nM osimertinib (osi) or osimertinib and 1000nM Cmp14 combo (OC) in HCC827* and HCC827GR6 cells. E. Drug synergy plots in HCC4006/HCC4006-OR and HCC827*/HCC827GR6 cells as measured using Combenefit software. Bliss synergy scores were calculated for each drug combination, Osimertinib (Osi) and Trametinib (Tram) or Osi alone in the absence or presence of Compound14 (Comp14) after 72 hours. Synergy is observed in HCC827GR6 cells when treated with Osi and Comp14. One representative experiment out of N=3 independent experiments is shown. F. Caspase assays performed in HCC827* and HCC827GR6 cells following across 100 hours of drug treatment. A combination of 100nM Osi with or without 30nM Tram was used were used in these assays. Cmp14 was used at 1000nM for all assays. Graphs represent fluorescent signals normalized to cellular confluency at each timepoint. G. Caspase assay images for PC9*/PC9-OR* cells at low and high OT concentrations (Figure 5B). While 100 nM Osi + 30 nM Tram induce apoptosis in PC9-OR* cells, many healthy surviving cells can still be seen in the wells. This drug tolerant population is effectively eliminated upon addition of 1000 nM Comp14 to OT. H. Immunoblot for SMARCA4, SMARCC1, GAPDH in PC9* /PC9-OR* upon 24hr OT and OT+Cmp14 treatments. I. Volcano plots reflecting differentially expressed genes across conditions indicated with significant genes up (red) and down (blue) indicated. J. Venn diagrams reflecting upregulated (left) and downregulated (right) genes overlapping between OT + Cmp14 vs OT compared to OT+Comp14 vs DMSO. K. Distance-to-TSS plot for ATAC gains (G) and losses (L) at resistance-associated accessible sites upon Cmp14 and Cmp14+OT treatment (vs DMSO). L. Venn diagram reflecting the overlap between downregulated genes in Cmp14 vs DMSO, OT+Cmp14 vs DMSO, and OT+Cmp14 vs OT to identify synergy-specific genes. M-N. Metascape analyses performed on up- and down-regulated genes from Cmp14 synergy and resistance reversal gene sets and Cmp14 synergy and sensitizing gene sets.

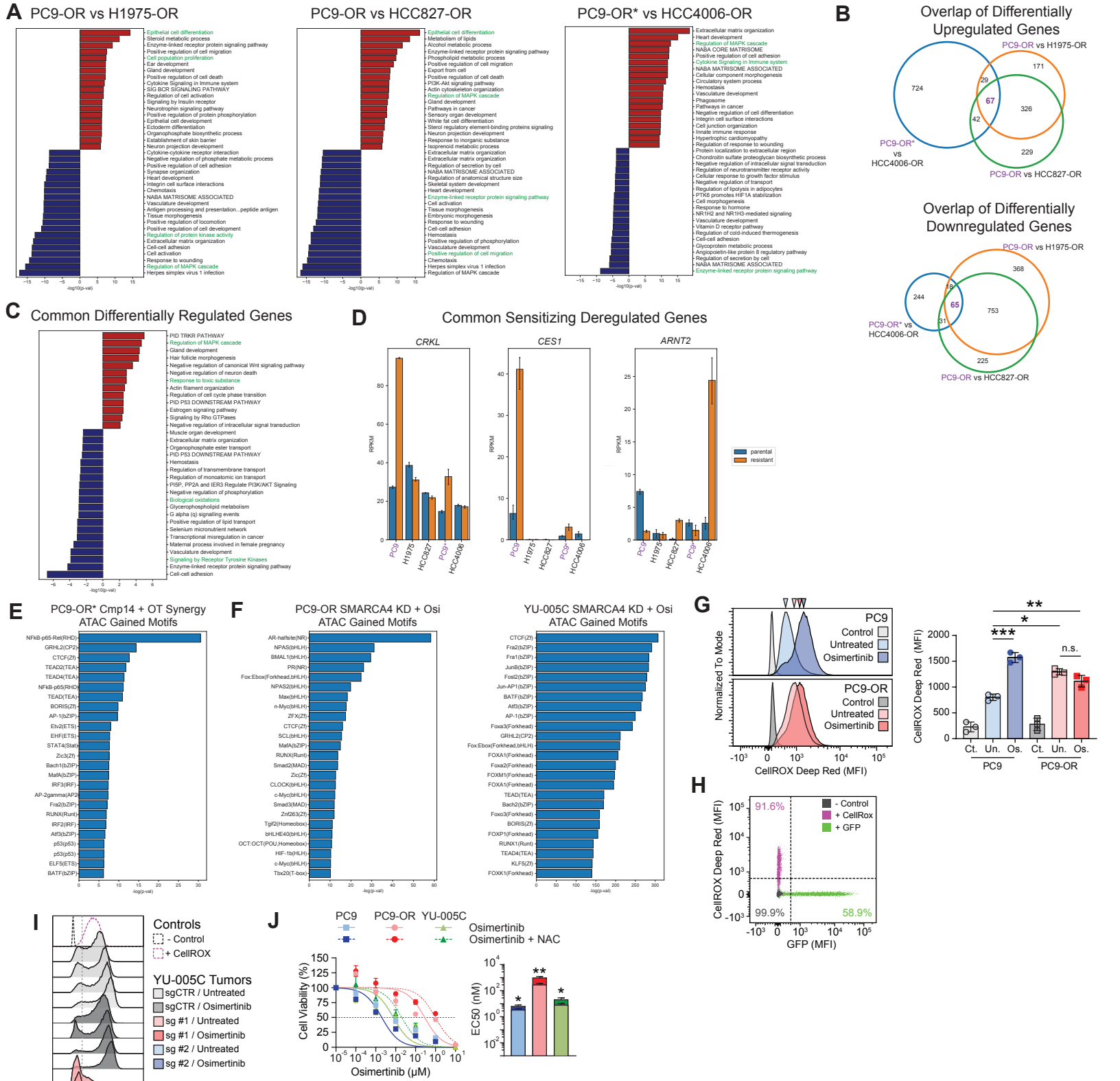

Figure S6. SMARCA4 attenuates reactive oxygen species in Osimertinib-resistant cells via NRF2 signaling, related to Figure 6. A. Metascape analyses performed on up- and down-regulated genes from the differential gene analysis of sensitizing vs non-sensitizing cell lines (Figure 6C). B. Venn diagram overlap of specific upregulated and downregulated genes amongst the three comparisons from Figure 6C. C. Metascape analysis from the overlap of common differentially expressed genes. Relevant pathways are highlighted. D. Bar graphs of key gene examples that are specifically upregulated and downregulated common to both PC9-OR* and PC9-OR showing RPKM values across cell lines. E-F. Motif analysis of gained ATAC sites attributed to Cmp14+OT treatment in PC9-OR* cells (E) and attributed to SMARCA4 knock down in PC9-OR and YU-005C cells (F). G. Flow cytometry using CellROXTM to measure ROS in PC9 & PC9-OR cells in the presence and absence of 750 nM osimertinib. Controls are from unstained cells Representative MFI profile of in RFP+/shRNA-containing CellROXTM+ cells (left panel). Quantification of three independent replicates (right panel). Paired t-test. H. Flow cytometry plot showing the controls and gating thresholds for GFP+ (Cas9/sgRNA+) and CellROXTM DeepRed+ cells used to analyze YU-005C tumors. The percentages show the fraction of cells from each sample in the quadrant. I. Flow cytometry MFI profiles for individual YU-005C tumors upon SMARCA4 knock-out as indicated. (-) Controls are YU-005C unstained cells and (+) CellROXTM Deep Red control is from stained YU-005C cells. J. Osimertinib dose-response curves for PC9, PC9-OR and YU-005C with or without 400 nM NAC (left panel). Bar graph of EC50 values. Mean ± SEM (right panel).***P<0.001, **P<0.01, *P<0.05.

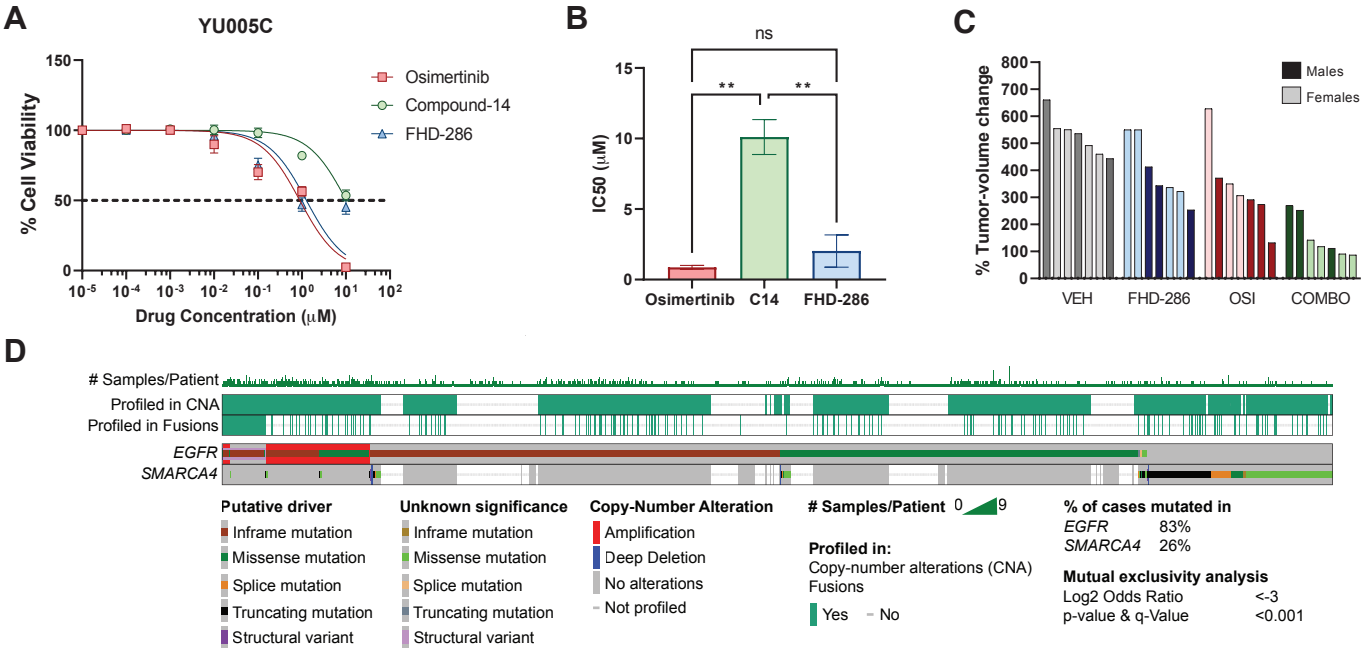

Figure S7. Pharmacological Inhibition of mSWI/SNF ATPase activity in a patient-derived model of EGFR-driven lung cancer, related to Figure 7. A. Single agent dose response curves for osimertinib, compound-14, and FHD-286 for YU-005C cells. N=4. The mean ± standard deviation is shown. B. Bar graph of individual IC50 values for YU-005C cells treated with osimertinib, compound-14, or FHD-286. The mean ± SEM is shown. Significance was calculated using the one-way repeated measures ANOVA test and Tukey's multiple comparisons test. ***P<0.001, **P<0.01, *P<0.05. C. Waterfall plot showing the % tumor volume change from treatment baseline of YU-005C cells injected subcutaneously in mice that were treated with either vehicle, osimertinib, FHD-286 or the combination of both. Females are indicated by a lighter shade and males are indicated by darker shaded boxes. D. Oncoprint of all lung adenocarcinomas from the GENIE Cohort v11.0-public with EGFR and/or SMARCA4 alterations. The data and the mutual exclusivity analysis was obtained from cBioPortal.