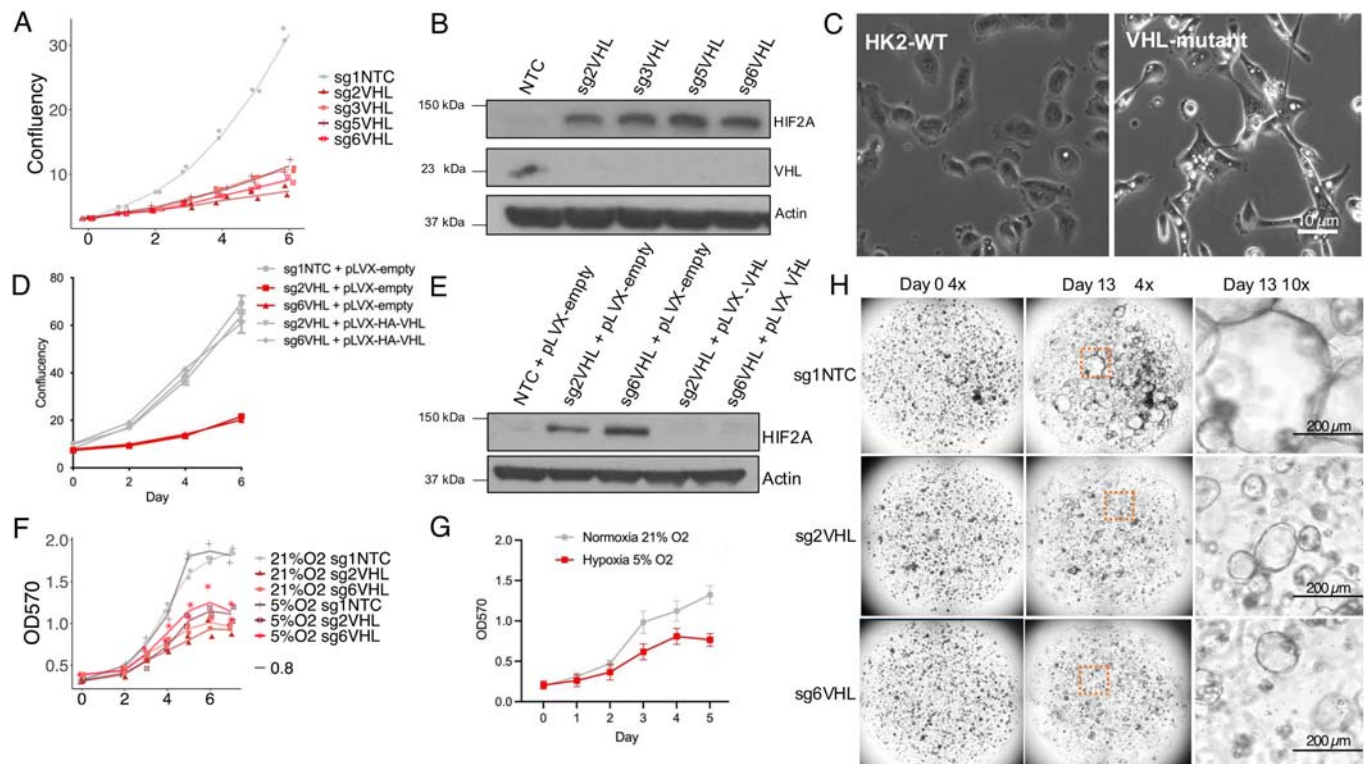


Expanded View Figures

**Figure EV1. VHL inactivation inhibits cell proliferation.**

(A) Proliferation of HK2 cells with or without VHL. $N = 2$ per condition. (mean and S.E.M.). (B) Western blot of HIF2A, VHL and Actin on HK2 cells with and without VHL. (C) Morphology of HK2 cells with and without VHL. (D) Proliferation of VHL mutant HK2 cells with and without VHL re-introduction. $N = 3$ per condition (mean and S.E.M.). (E) Western blot of HIF2A on VHL mutant HK2 cells with and without VHL re-introduction. (F) Proliferation of HK2 cells with and without VHL under 5% or 21% O₂ culture conditions. $N = 2$ per condition (mean and S.E.M.). (G) Representative images of human renal epithelial organoids with and without VHL. $N = 4$ replicates per condition. (H) Human renal epithelial organoid proliferation under DMSO or DMOG (3 mM) treatment. Source data are available online for this figure.

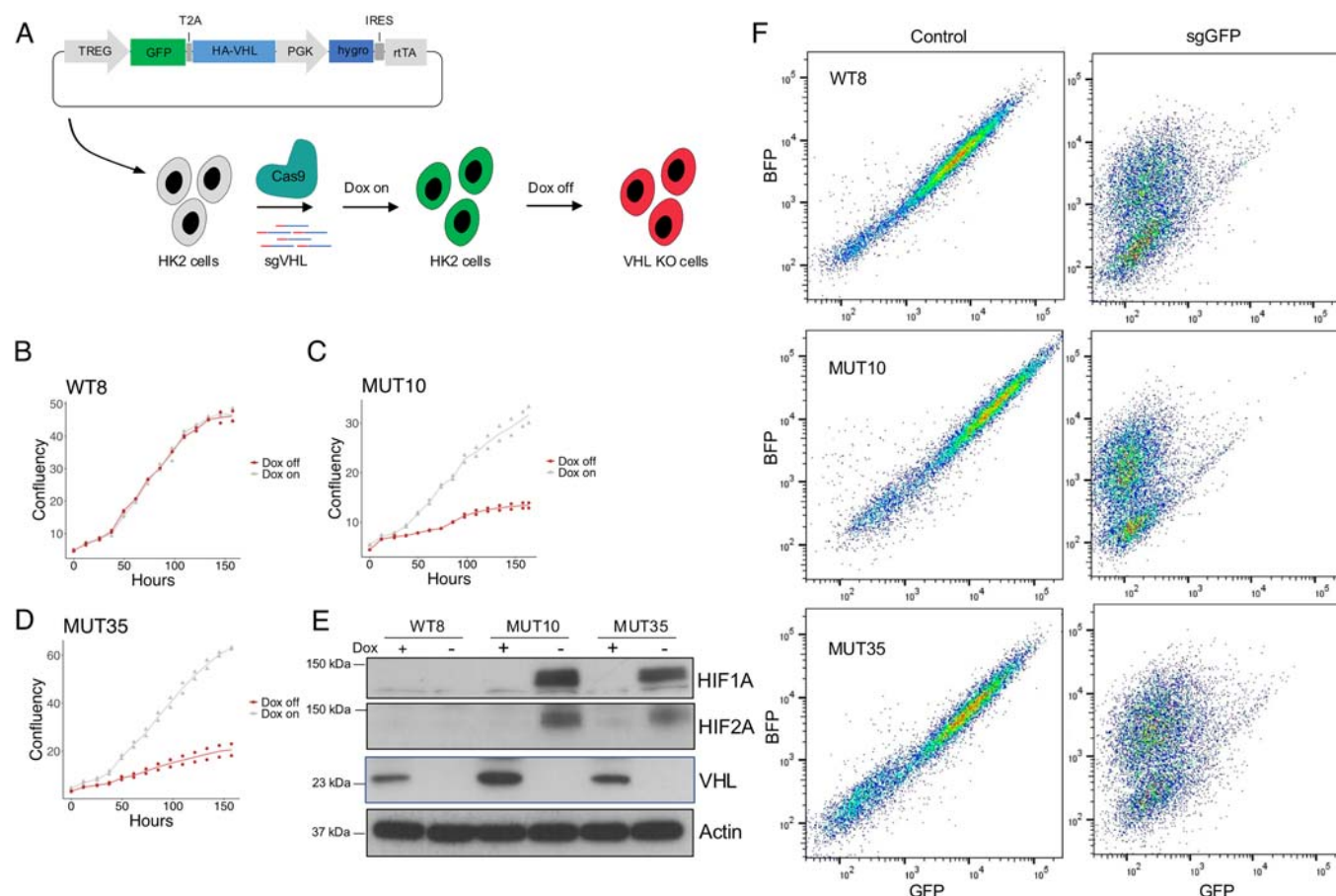


Figure EV2. Establishment of models with doxycycline-controllable VHL expression.

(A) Schematic of doxycycline (dox) inducible VHL re-introduction into VHL mutant (MUT10 and MUT35) and wild-type control (WT8) clones. (B–D) Proliferation of WT8 (B), MUT10 (C) and MUT35 (D) cells with and without dox. $N = 2$ replicates per condition (mean and S.E.M.). (E) Western blot of HIF1A, HIF2A, VHL and Actin on WT8, MUT10 and MUT35 cells with and without dox. (F) Cas9 editing efficiency tested on WT8, MUT10 and MUT35 cells by a reporter plasmid using fluorescence-activated cell sorting. Source data are available online for this figure.

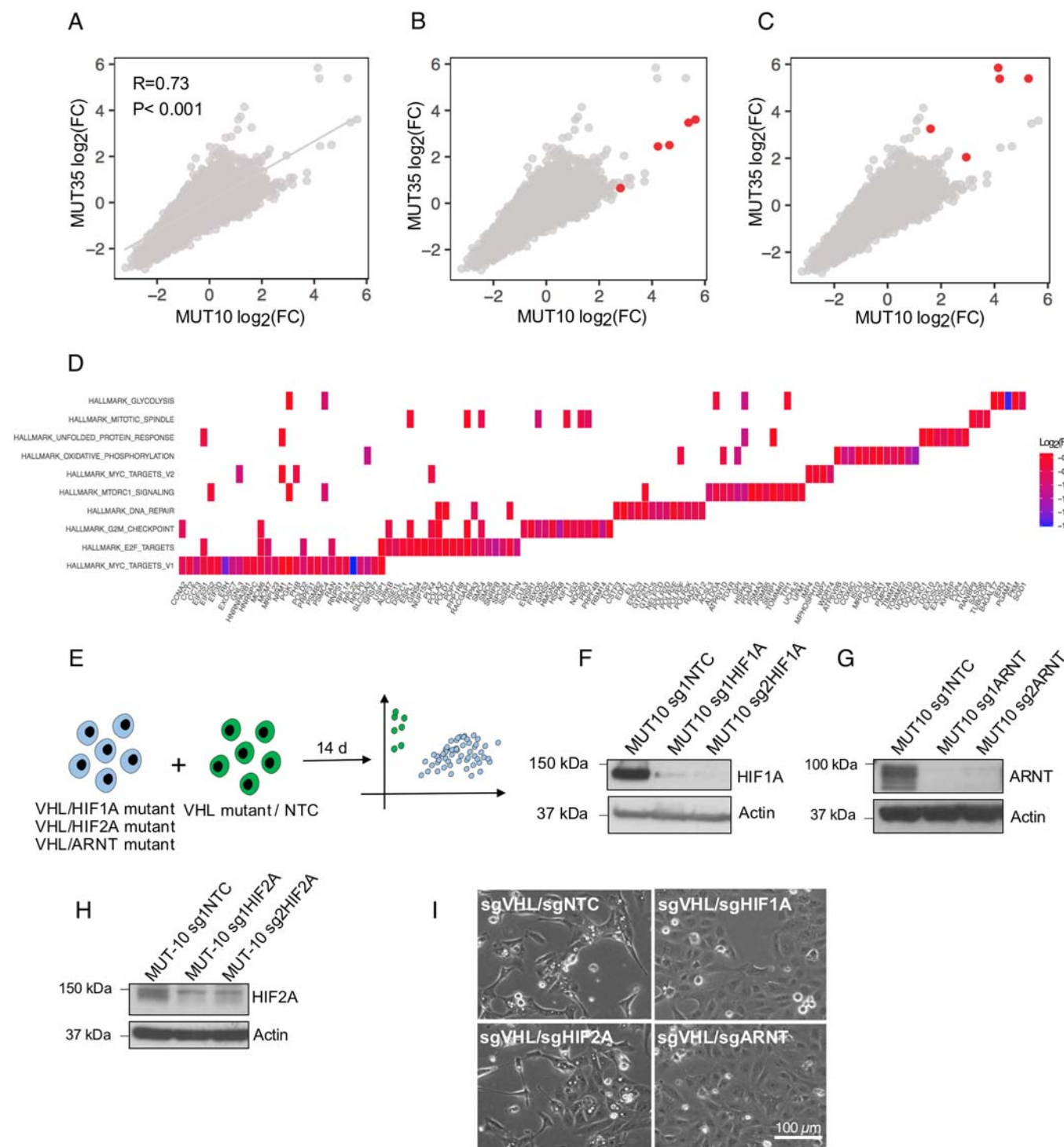


Figure EV3. HIF1A and ARNT inhibit proliferation upon VHL inactivation.

(A) CRISPR/Cas9-based genome wide screen data. sgRNA abundance on day 28 relative to start of the assay in VHL mutant clones MUT10 and MUT35. R, Pearson's correlation coefficient. (B, C) As in (A) with sgRNAs targeting genes of interest highlighted in red: HIF1A in (B) and ARNT in (C). (D) Pathway enrichment analysis on the top 500 genes the sgRNAs of which are depleted over time in MUT10 and MUT35 cells using the Cancer Hallmarks gene sets. (E) A schematic of the competitive proliferation assay. VHL-HIF1A, VHL-HIF2A and VHL-ARNT double mutant cells (BFP labelled) competed against VHL-NTC single mutant cells (GFP labelled). (F-H) Western blot of HIF1A, ARNT and HIF2A on MUT10 cells with and without HIF1A, ARNT or HIF2A inactivation, respectively. (I) Morphology of VHL-NTC, VHL-HIF1A, VHL-HIF2A and VHL-ARNT cells. Source data are available online for this figure.

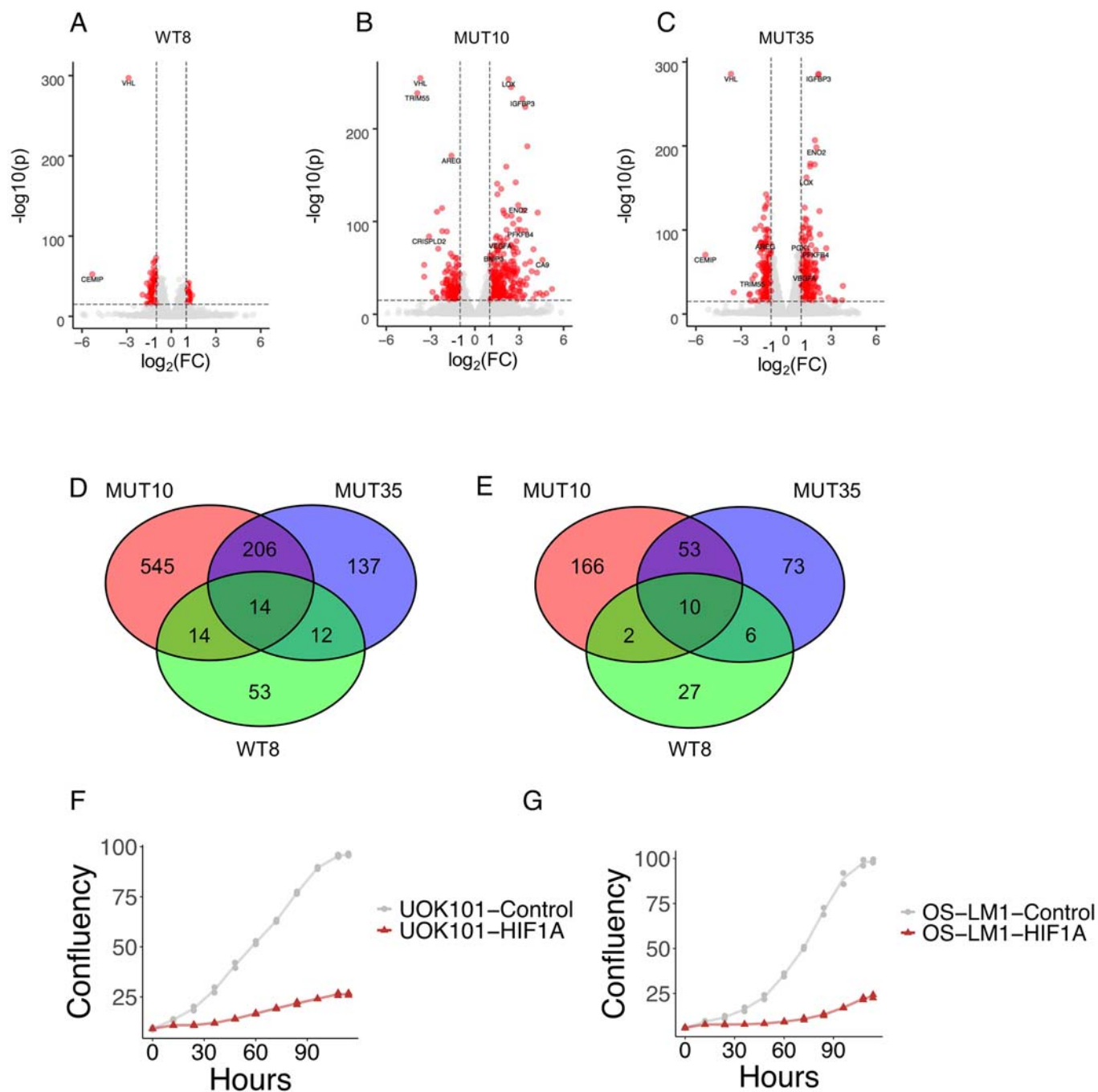


Figure EV4. HIF1A inhibits mitochondrial function.

(A–C) Differential gene expression analysis by RNA-seq. WT8, MUT10 and MUT35 cells, dox off compared to dox on. Adjusted *P* value and fold change determined by DESeq2, using the Wald test to test the significance. *N* = 4 replicates per condition. (D) Venn diagram of upregulated genes upon dox withdrawal for WT8, MUT10 and MUT35 cells. (E) Venn diagram of downregulated genes upon dox withdrawal for WT8, MUT10 and MUT35. (F) Proliferation of UOK101 ccRCC cells with and without HIF1A cDNA expression. *N* = 2 replicates per condition (mean and S.E.M.). (G) Proliferation of OS-LM1 ccRCC cells with and without HIF1A cDNA expression. *N* = 2 replicates per condition (mean and S.E.M.). Source data are available online for this figure.

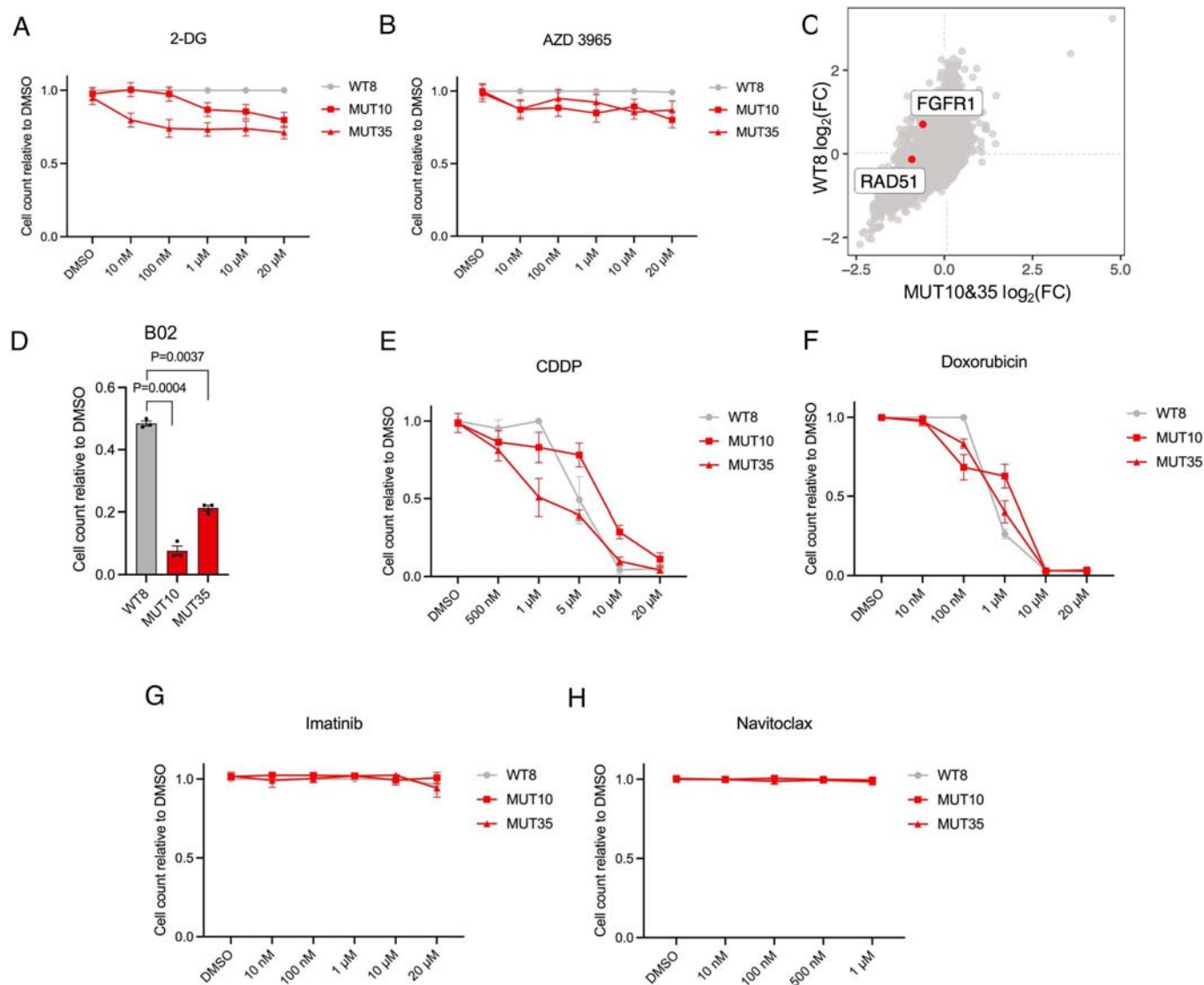


Figure EV5. VHL mutant cells show druggable genetic vulnerabilities.

(A, B) Cell counts on day 5 relative to DMSO control group under 2-DG (10 nM, 100 nM, 1 μ M, 10 μ M, 20 μ M) and AZD3965 (10 nM, 100 nM, 1 μ M, 10 μ M, 20 μ M) treatments. $N = 4$ per condition (mean and SD). (C) CRISPR-Cas9 screen data. FGFR1 and RAD51 gene beta score distribution in VHL WT and mutant cells. (D) Cell counts on day 5 relative to DMSO control group under B02 (10 μ M). $N = 3$ replicates per condition (mean and SD). Paired t test. (E-H) Cell counts on day 5 relative to DMSO control group under CDDP (10 nM, 100 nM, 1 μ M, 10 μ M, 20 μ M), Doxorubicin (10 nM, 100 nM, 1 μ M, 10 μ M, 20 μ M), Imatinib (10 nM, 100 nM, 1 μ M, 10 μ M, 20 μ M) and Navitoclax (10 nM, 100 nM, 1 μ M, 10 μ M, 20 μ M). $N = 3$ replicates per condition (mean and SD). Source data are available online for this figure.

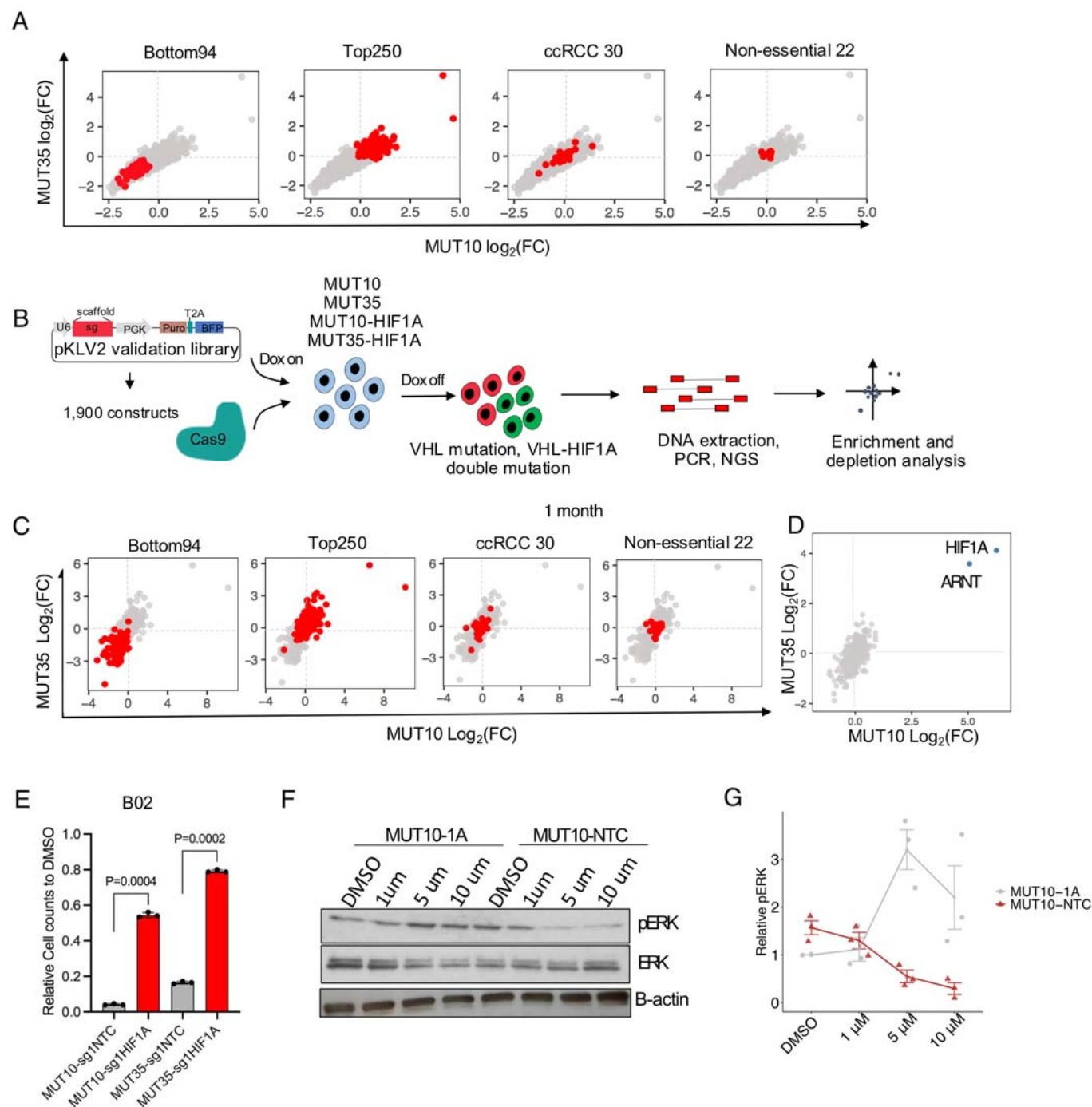


Figure EV6. CRISPR-Cas9 validation screen.

(A) Genes selected for the validation screen. Distribution of beta scores in the genome-wide CRISPR-Cas9 screen data set. 94 genes the constructs of which were specifically depleted in *VHL* mutant cells, 250 the constructs of which were specifically enriched in *VHL* mutant cells, 30 frequently mutated ccRCC genes and 22 non-essential control genes. (B) Schematic of the validation screen on MUT10, MUT35, MUT10-sgHIF1A and MUT35-sgHIF1A cells. (C) CRISPR-Cas9-based validation screen data. Gene level construct abundance relative to start of the assay in MUT10 and MUT35 cells. Gene sets of interest highlighted in red. (D) CRISPR-Cas9-based validation screen data, doxycycline withdrawal before sgRNA transduction. Gene level construct abundance relative to start of the assay in MUT10 and MUT35 cells. (E) Cell counts of B02 (10 μ M) treated cells on day 5 relative to DMSO control group. $N = 3$ replicates per condition (mean and SD). Student's t test. (F) WB of MUT1-1A and MUT10-NTC treated with DMSO, 1 μ M, 5 μ M, 10 μ M Pazopanib for pERK, total ERK and beta-actin. (G) Quantification of relative pERK at different treatment concentration at indicated cell population. $N = 3$ replicates per condition (mean and S.E.M.). Source data are available online for this figure.

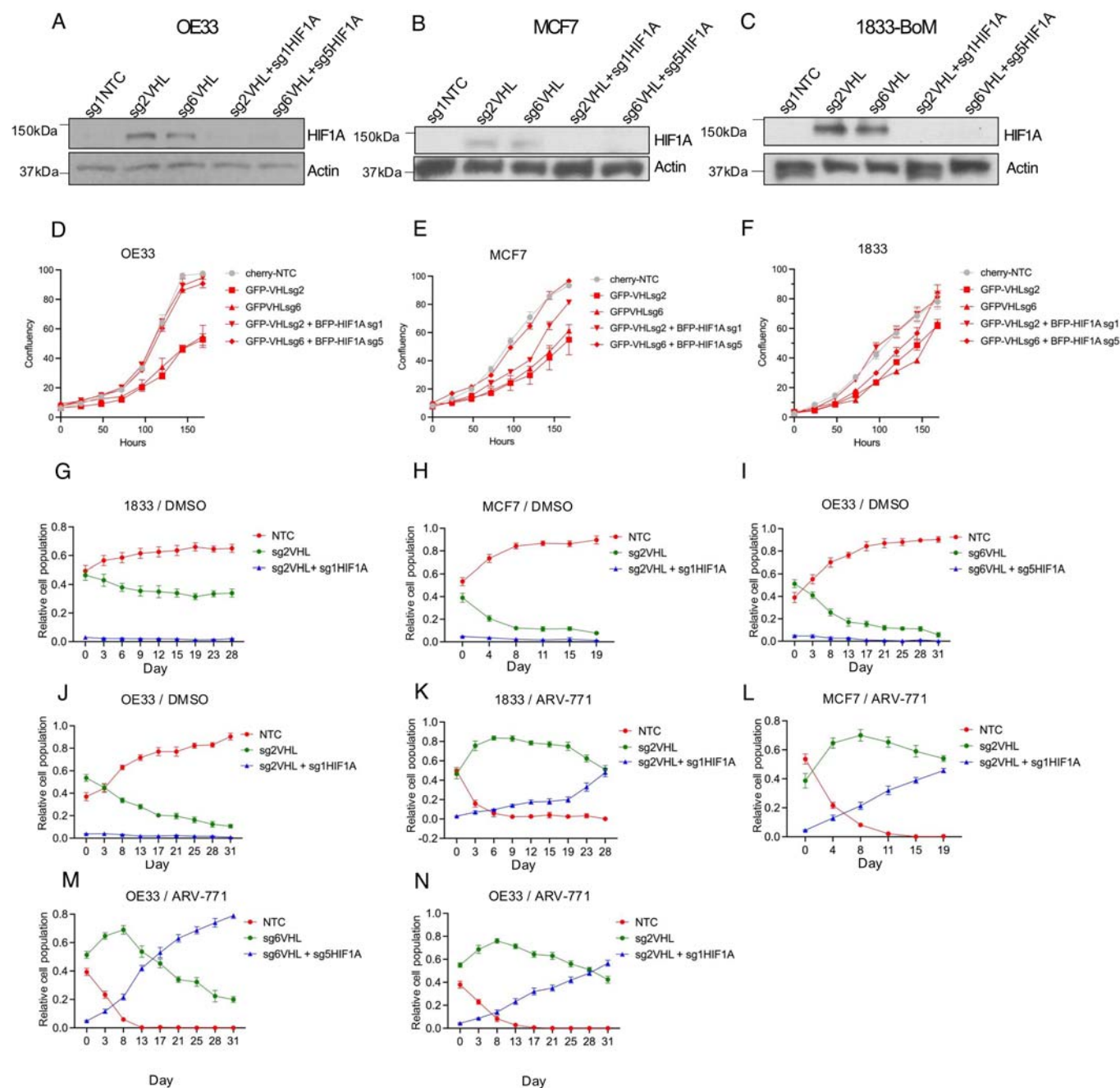


Figure EV7. HIF1A loss-mediated escape from PROTAC-induced growth inhibition in cancer.

(A–C) Western blot of HIF1A and Actin on WT (NTC), VHL mutant (VHLsg2, VHLsg6) and VHL-HIF1A double mutant (VHLsg2/HIF1Asg1, VHLsg6/HIF1Asg5) OE33 (A), MCF7 (B) and 1833-BoM (C) cells. (D–F) Incubates proliferation of OE33, MCF7 and 1833 under different genotype. $N = 3$. (G–N) FACS-based quantification of the relative abundances of different cell populations in competition assays. 1833-BoM cells: 500 nM ARV-771; MCF7 cells: 200 nM ARV-771; OE33 cells: 400 nM ARV-771. $N = 3$ for each condition and timepoint (mean and SD). Source data are available online for this figure.