

Title: Supplementary Data 1

Description: FHSC14 GeCKO v2 pilot genome scale sgRNA screen analysis and results. Top 500 genes enriched in the CIS-ETO vs. P0 condition are shown (ranked by positive adjusted p-value). Statistical analyses were performed by differential abundance analysis as described in CRISPRko Screen Analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 2

Description: Gene composition and sgRNA sequences in the focused sgRNA library used for focused CRISPR deletion screens in this study.

Title: Supplementary Data 3

Description: NCI109B focused sgRNA screen gene-level analysis and results. Statistical analyses were performed by differential abundance analysis as described in CRISPRko Screen Analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 4

Description: Enriched sgRNAs ($\log_2FC > 2$, $FDR < 0.05$) from the NCI109B CRISPR deletion screen. Statistical analyses were performed by differential abundance analysis as described in CRISPRko Screen Analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 5

Description: sgRNA sequences used in this study for isogenic model generation

Title: Supplementary Data 6

Description: List of known USP22 mutations in SCLC

Title: Supplementary Data 7

Description: FHSC14 global proteomics analysis. Differentially-expressed proteins ($FDR < 0.05$, $\log_2FC > 0.5$ or $\log_2FC < -0.5$) of sgUSP22 vs. sgCtrl in the CIS-ETO and SAL conditions. Statistical analyses were performed by linear regression model analysis as described in Global proteomics and phospho-proteomics analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 8

Description: NCI109B global proteomics analysis. Differentially-expressed proteins ($FDR < 0.05$, $\log_2FC > 0.5$ or $\log_2FC < -0.5$) of sgUSP22 vs. sgCtrl in the CIS-ETO and SAL conditions. Statistical analyses were performed by linear regression model analysis as described in Global proteomics and phospho-proteomics analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 9

Description: RNA-seq Enrichr pathway analyses: MSigDB Hallmark. Statistical analyses were performed by gene enrichment analysis and fisher's exact test. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 10

Description: RNA-seq Enrichr pathway analyses: GO Biological Process. Statistical analyses were performed by gene enrichment analysis and fisher's exact test. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 11

Description: RNA-seq Enrichr pathway analyses: MSigDB WikiPathway. Statistical analyses were performed by gene enrichment analysis and fisher's exact test. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 12

Description: RNA-seq Enrichr pathway analyses: MSigDB KEGG. Statistical analyses were performed by gene enrichment analysis and fisher's exact test. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 13

Description: FHSC14 Proteomics vs RNA-seq to identify candidate non-histone substrates of USP22. Genes downregulated at the protein level ($\text{Log2FC} < -0.5$, $\text{FDR} < 0.05$ in GLBL Proteomics dataset) but not the RNA level ($\neq \text{Log2FC} < 0$ with $\text{FDR} < 0.05$ in RNA-seq dataset) for each treatment condition in FHSC14 sgUSP22 vs sgCtrl tumors

Title: Supplementary Data 14

Description: NCI109B Proteomics vs RNA-seq to identify candidate non-histone substrates of USP22. Genes downregulated at the protein level ($\text{Log2FC} < -0.5$, $\text{FDR} < 0.05$ in GLBL Proteomics dataset) but not the RNA level ($\neq \text{Log2FC} < 0$ with $\text{FDR} < 0.05$ in RNA-seq dataset) for each treatment condition in FHSC14 sgUSP22 vs sgCtrl tumors

Title: Supplementary Data 15

Description: FHSC14 CUT&Tag analysis - H2AK119ub. Differential peaks (FDR<0.05) of sgUSP22 vs. sgCtrl in the CIS-ETO and SAL conditions. Peaks with Log2FC > 0 and FDR < 0.05 are enriched in sgUSP22. Statistical analyses were performed by differential peak analysis as described in CUT&Tag section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 16

Description: FHSC14 CUT&Tag RNA-seq integrated analysis - H2AK119ub. Genes with enriched H2AK119ub peaks (Log2FC > 0, FDR < 0.05) with concurrent downregulated RNA (Log2FC < 0, FDR < 0.05) in sgUSP22 vs. sgCtrl in SAL and CIS-ETO condition.

Title: Supplementary Data 17

Description: FHSC14 phospho-proteomics analysis. Differentially-expressed phospho-sites (FDR<0.05, Log2FC > 0.5 or Log2FC < -0.5) of sgUSP22 vs. sgCtrl in the CIS-ETO and SAL conditions. Statistical analyses were performed by linear regression model analysis as described in Global proteomics and phospho-proteomics analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 18

Description: NCI109B phospho-proteomics analysis. Differentially-expressed phospho-sites (FDR<0.05, Log2FC > 0.5 or Log2FC < -0.5) of sgUSP22 vs. sgCtrl in the CIS-ETO and SAL conditions. Statistical analyses were performed by linear regression model analysis as described in Global proteomics and phospho-proteomics analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.

Title: Supplementary Data 19

Description: JHU-LX33 phospho-proteomics analysis. Differentially-expressed phospho-sites (unadjusted p-value < 0.01, Log2FC > 0.5 or Log2FC < -0.5) of USP22 vs. Empty vector control in the CIS-ETO and SAL conditions. Statistical analyses were performed by linear regression model analysis as described in Global proteomics and phospho-proteomics analyses section in Methods. P-values are adjusted using Benjamini-Hochberg FDR procedure.