

Supplemental information

Heterozygous mutations in valosin-containing protein (VCP) and resistance to VCP inhibitors

Prabhakar Bastola¹, Rabeya Bilkis², Cristabelle De Souza^{2,3}, Kay Minn⁴, and Jeremy Chien^{3*}

¹Department of Laboratory Medicine, University of California San Francisco, 185 Berry Street, San Francisco, 94143, California, USA

²Division of Molecular Medicine, University of New Mexico Health Sciences Center, 915 Camino de Salud NE, Albuquerque, 87131, New Mexico, USA

³Department of Biochemistry and Molecular Medicine, University of California, Davis. 2700 Stockton Blvd. Sacramento, 95817, California, USA

⁴Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First Street SW, Rochester, 55905, Minnesota, USA

*Corresponding author; jrchien@ucdavis.edu; ORCID: 0000-0003-4744-8374

Supplemental information

Complete results of Metascape analysis are available at the following link:

<https://osf.io/uzdbw/>

Complete results of GSEA analysis are available at the following link:

<https://osf.io/uzdbw/>

Complete results of Connectivity Map analysis are available at the following link:

<https://osf.io/uzdbw/>

Complete results of Mutation analysis from RNA sequencing of resistant cells are available at the following link:

<https://osf.io/uzdbw/>

Supplementary Table [S1](#)

Supplementary Table [S2](#)

Supplementary Table [S3](#)

Supplementary Table [S4](#)

Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure S3

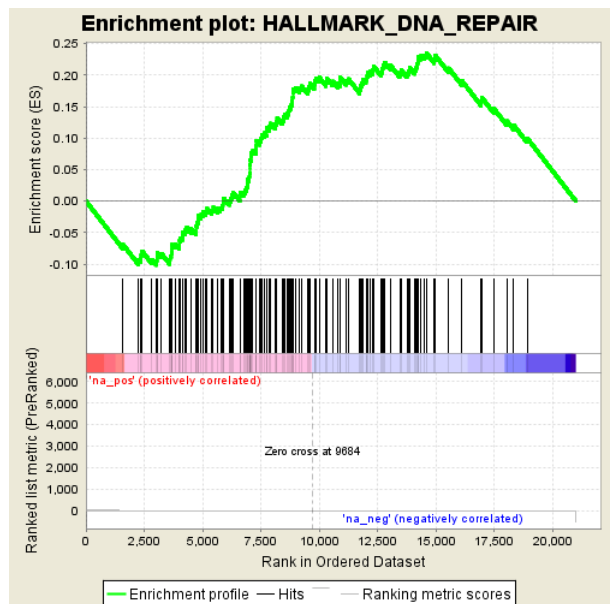
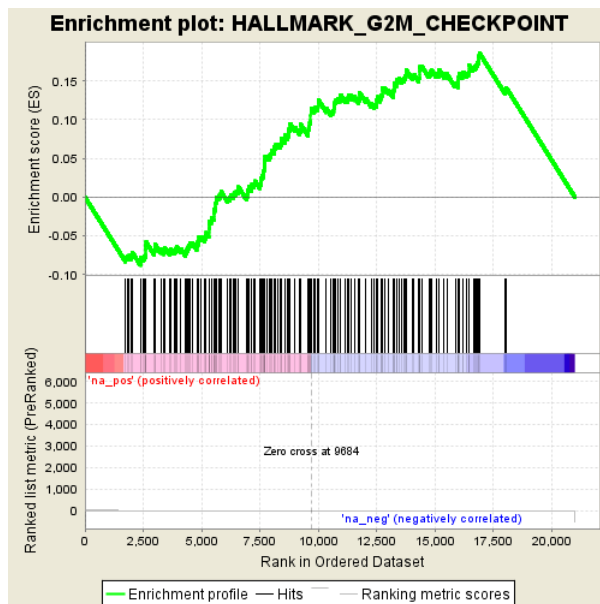
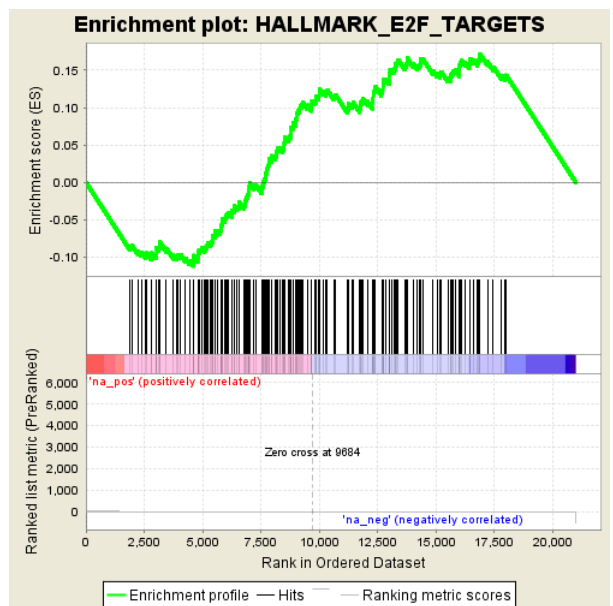
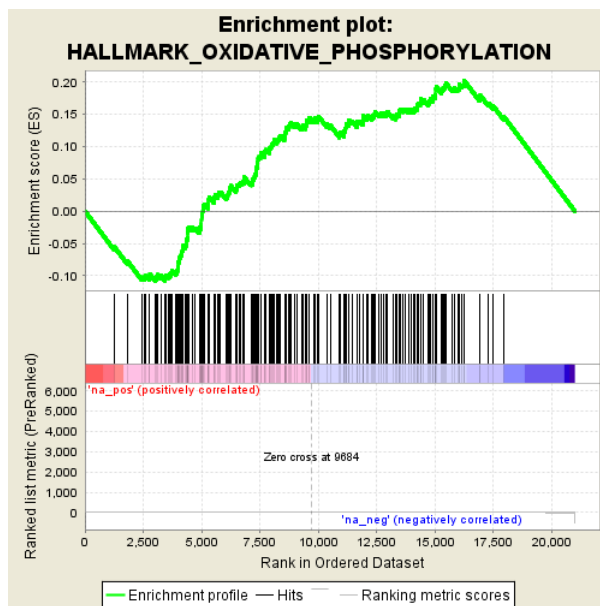


Figure S1: Hallmark gene sets positively enriched in resistant cells treated with CB-5083. Log2-transformed, ranked fold-change in gene expression between CB-5083-treated and DMSO-treated OVSAHO cells (CB/DMSO) is used for GSEA analysis. Na_pos = Genes upregulated by CB-5083. Na_neg = genes downregulated by CB-5083. Complete results of GSEA are available at the following link: <https://osf.io/uzdbw/>

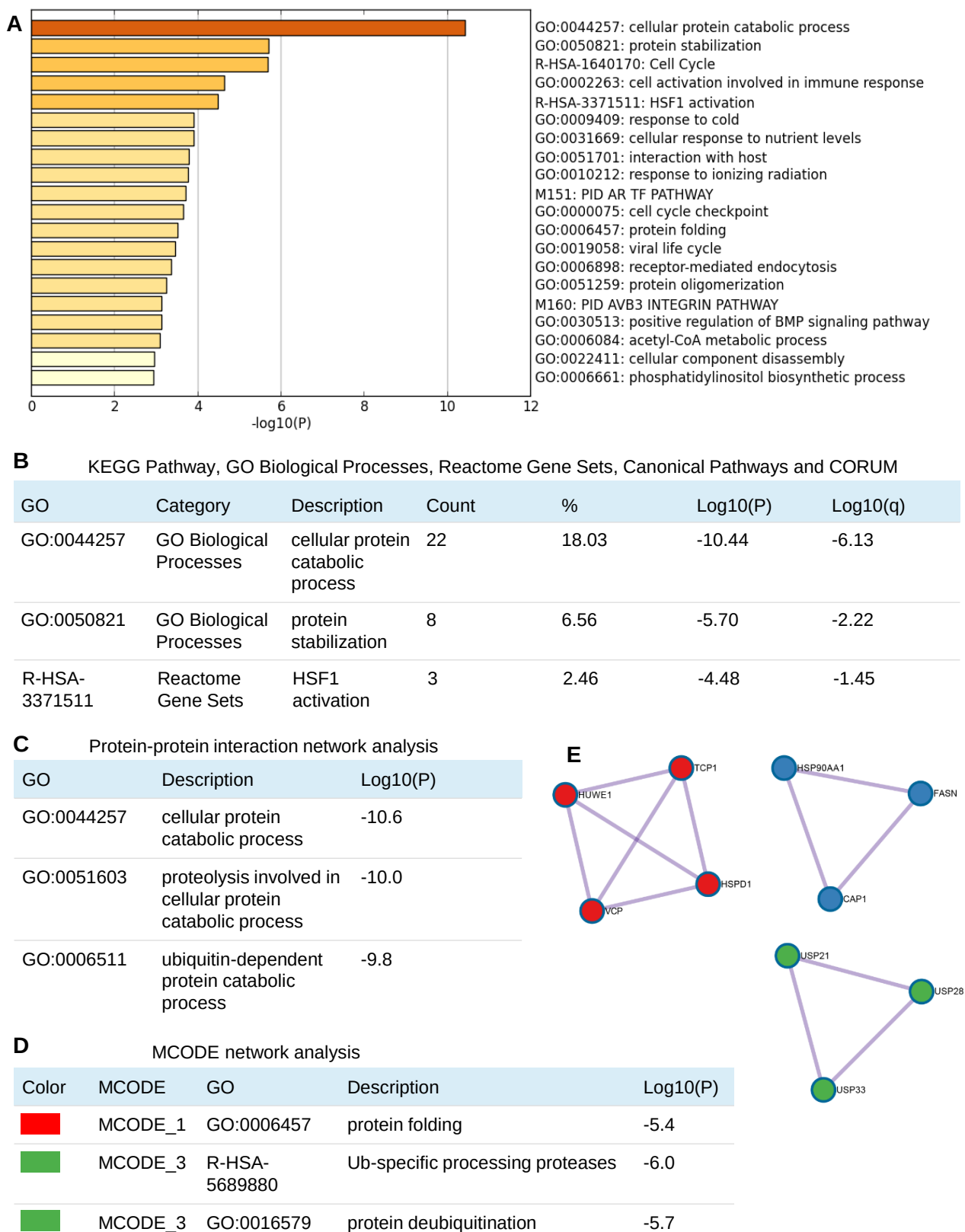


Figure S2: Metascape analysis of mutated genes in CB-5083-resistant cells. **(A-B)** Gene Ontology (GO) Biological Processes, Reactome Gene Sets (R) and Canonical Pathways (M) significantly enriched by the mutated genes observed in resistant cells. **(C)** Protein-protein interaction networks enriched in mutated genes. **(D-E)** MCODE network analysis of genes mutated in resistant cells. Complete results of the Metascape analysis are available at the following link: <https://osf.io/uzdbw/>

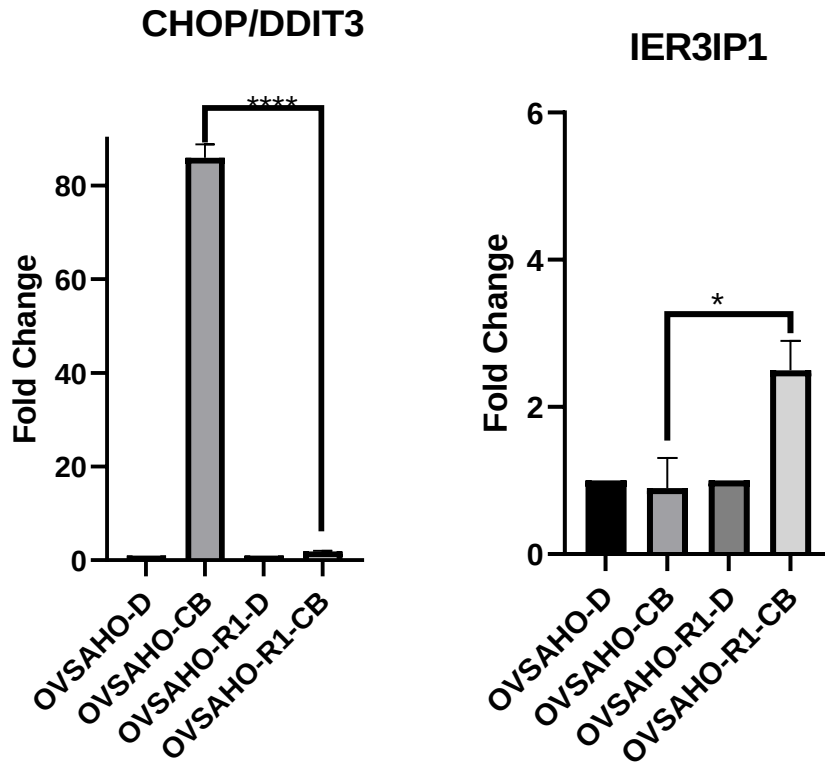


Figure S3: To assess the effect of CB-5083 treatment on OVSAHO Parental and OVSAHO-R1 cells, we performed RT-qPCR on both parental and resistant cell lines upon 6 hours of treatment with 5 μ M CB-5083 (CB) or vehicle DMSO (D). Data are shown as mean $\pm$ SEM. Statistics were performed with Student's t-test, and $p \leq 0.05$ was considered significant. * = $p \leq 0.05$, **** = $p \leq 0.0001$.

The following primers were used in the study:

CHOP

Forward	TGTTAAAGATGAGCGGGTGG
Reverse	CCTTCTTGAACACTCTCTCCTC

IER3IP1

Forward	CCTTTACCCTGTACTCACTGC
Reverse	CTGTGATTTAATTCCCGGCTC

GAPDH

Forward	GTCTCCTCTGACTTCAACAGCG
Reverse	ACCACCCTGTTGCTGTAGCCAA

Table S1.

The results are available at the following link:

<https://osf.io/uzdbw/>

The results are obtained from variant analysis of RNA sequencing data from parental OVSAHO vs resistant OVSAHO using CLC Genomic Workbench ver. 10. Only non-synonymous variants from the sequencing results at base positions with at least 25X coverage are shown.

Supplementary Table [S1](#)

<https://osf.io/zaw79/>

Supplementary Table [S2](#)

<https://osf.io/r4y7v/>

Supplementary Table [S3](#)

<https://osf.io/nksmx/>

Supplementary Table [S4](#)

<https://osf.io/84e9t/>