

**Title: Multiple components of protein homeostasis pathway can be targeted to produce drug synergies with VCP inhibitors in ovarian cancer.**

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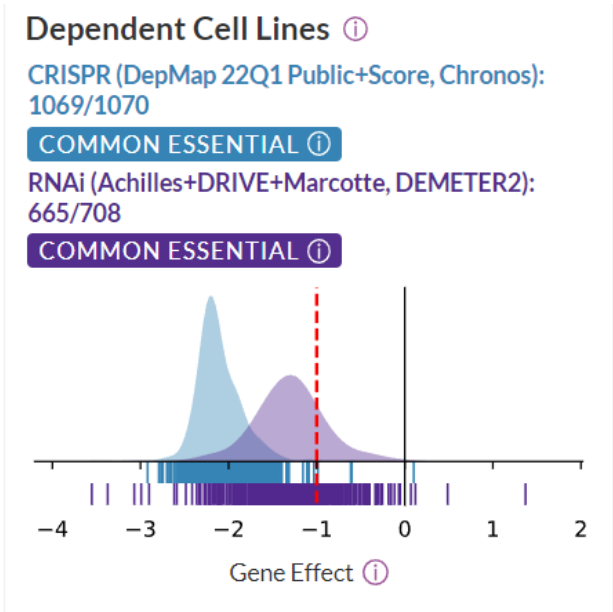
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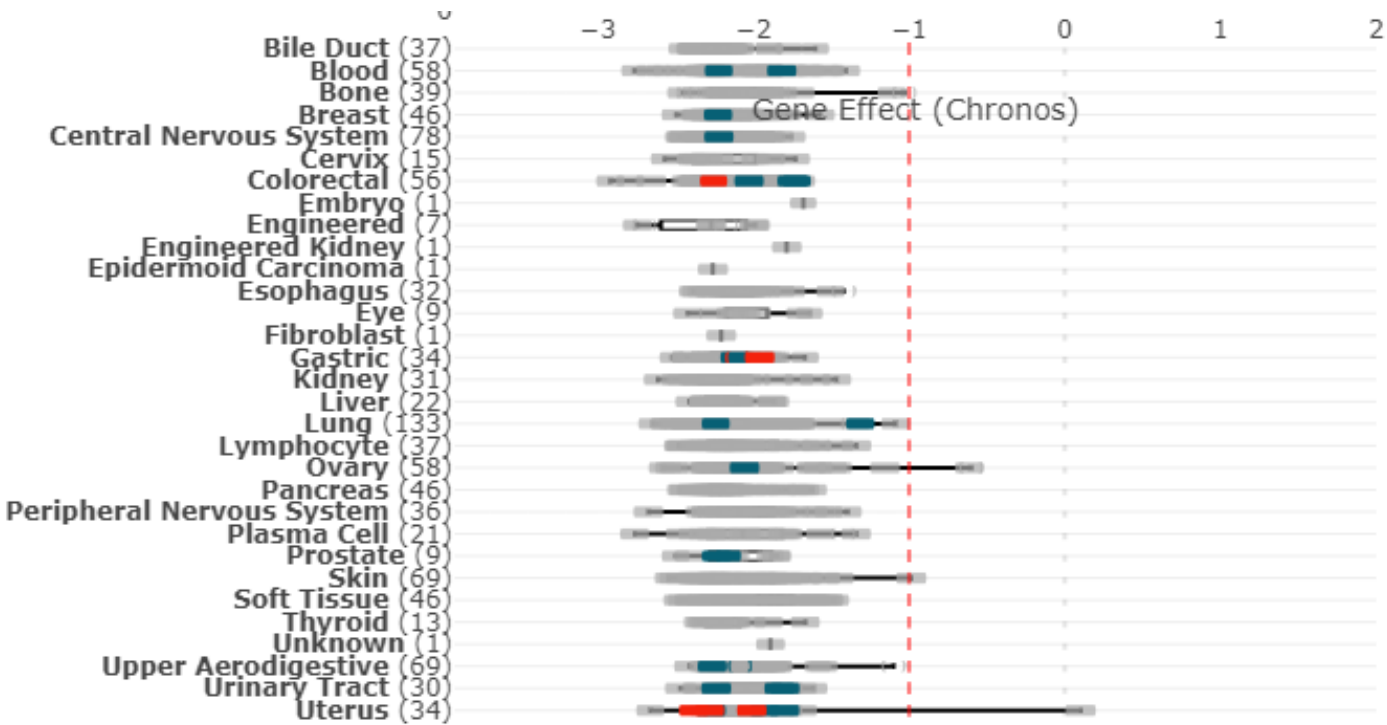
<sup>3</sup>Department of Biochemistry and Molecular Medicine, University of California, Davis, CA 95817.

Figure S1

A



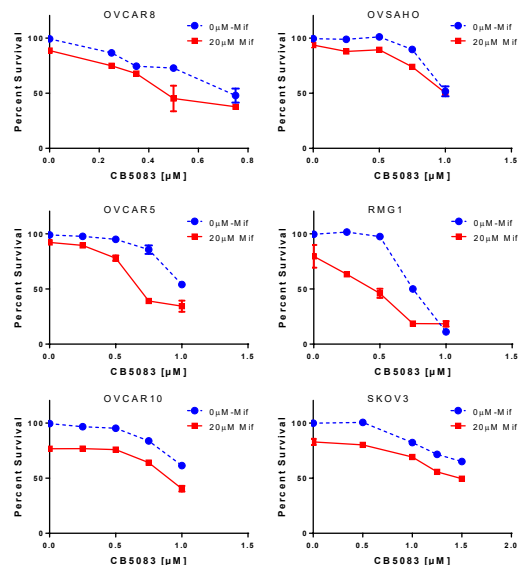
B



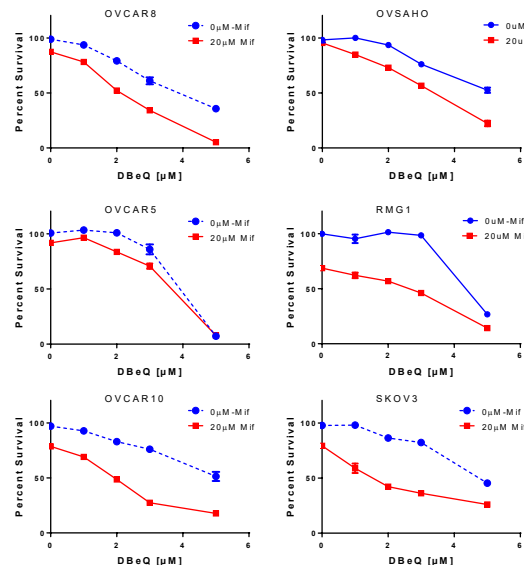
A:Gene effect depicted from CRISPR and RNAi screens . A lower gene effect score suggest that the gene is more likely to be essential in the tested cell line. Score of 0 represents non-essential, while score of -1 represents a median for all common essential genes. B: Gene effect score depicted from CRISPR screens in different cancer cell lines, including ovarian cancer cells (ovary). Both data sets was obtained from the DepMap Portal on April 02, 2022.

Figure S2

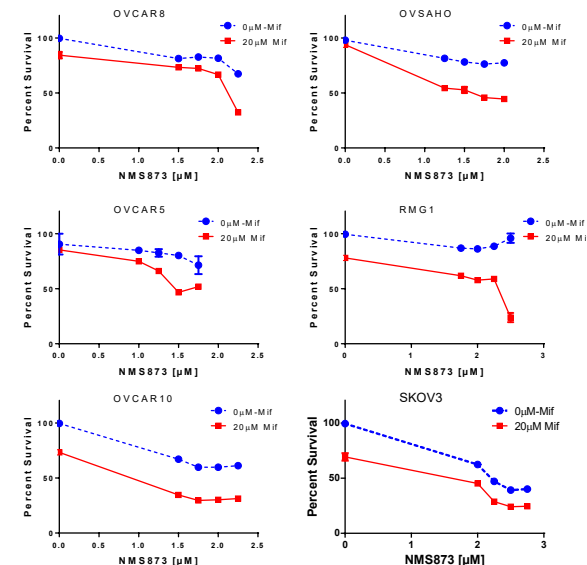
A



B

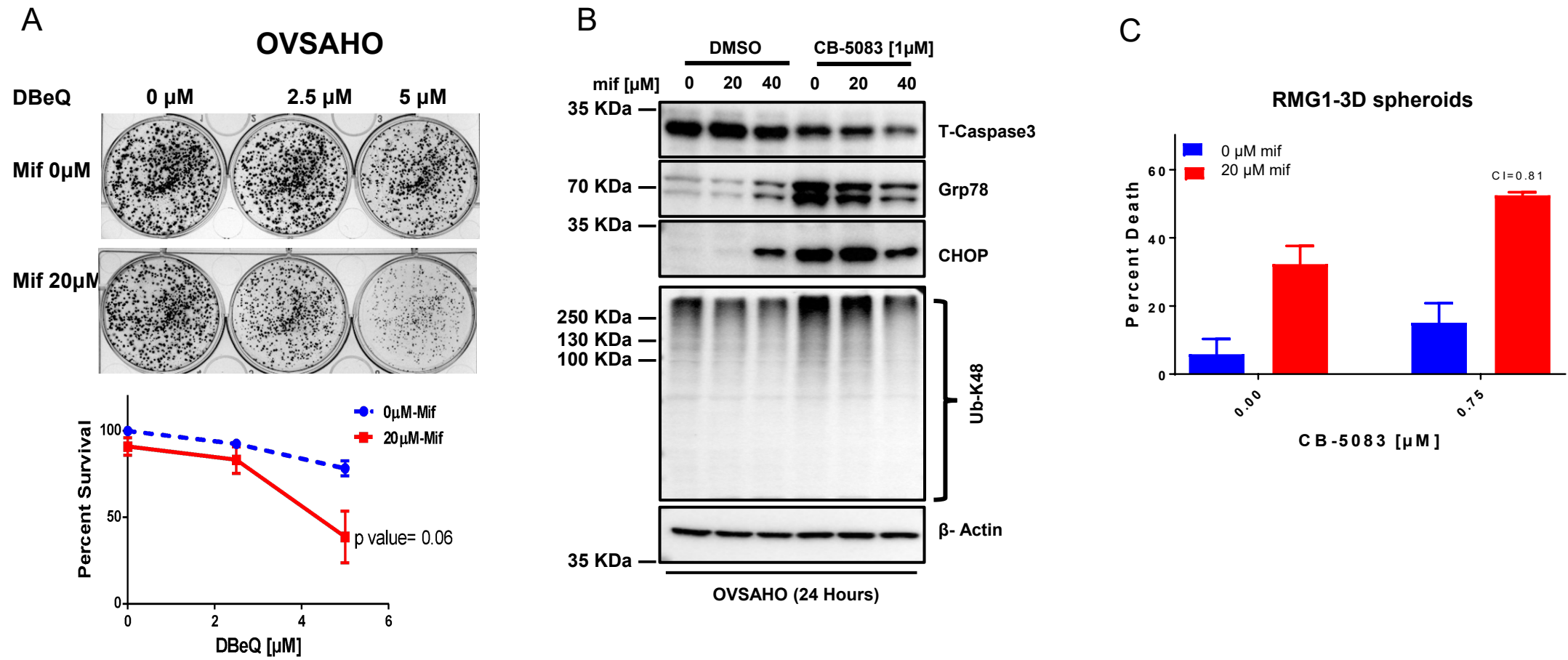


C



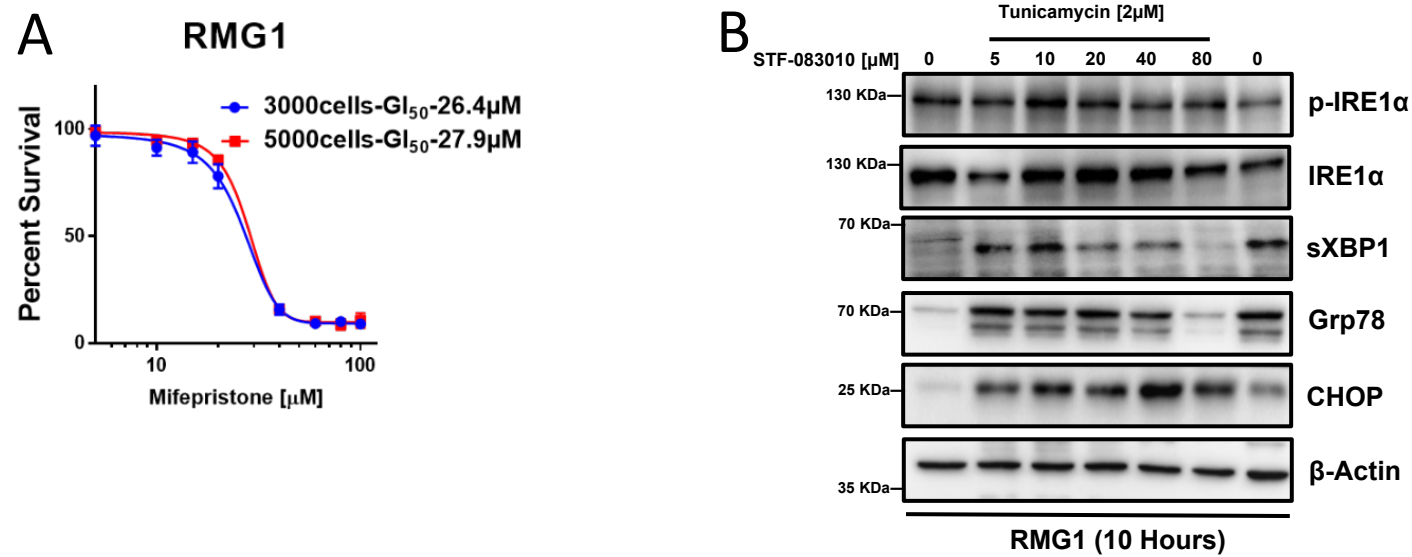
A: Dose response following treatment with vehicle, indicated concentration of CB-5083 with or without 20  $\mu$ M mifepristone in indicated ovarian cancer cells. B: Same as A, but cells were incubated with indicated concentrations of DBE-Q instead of CB-5083. C: Same as A, but cells were incubated with indicated concentrations of NMS-873. Dose-response curves were generated using GraphPad Prism. Every point in the dose-response curve represents Mean  $\pm$  SEM taken from three technical replicates for all cell lines.

Figure S3



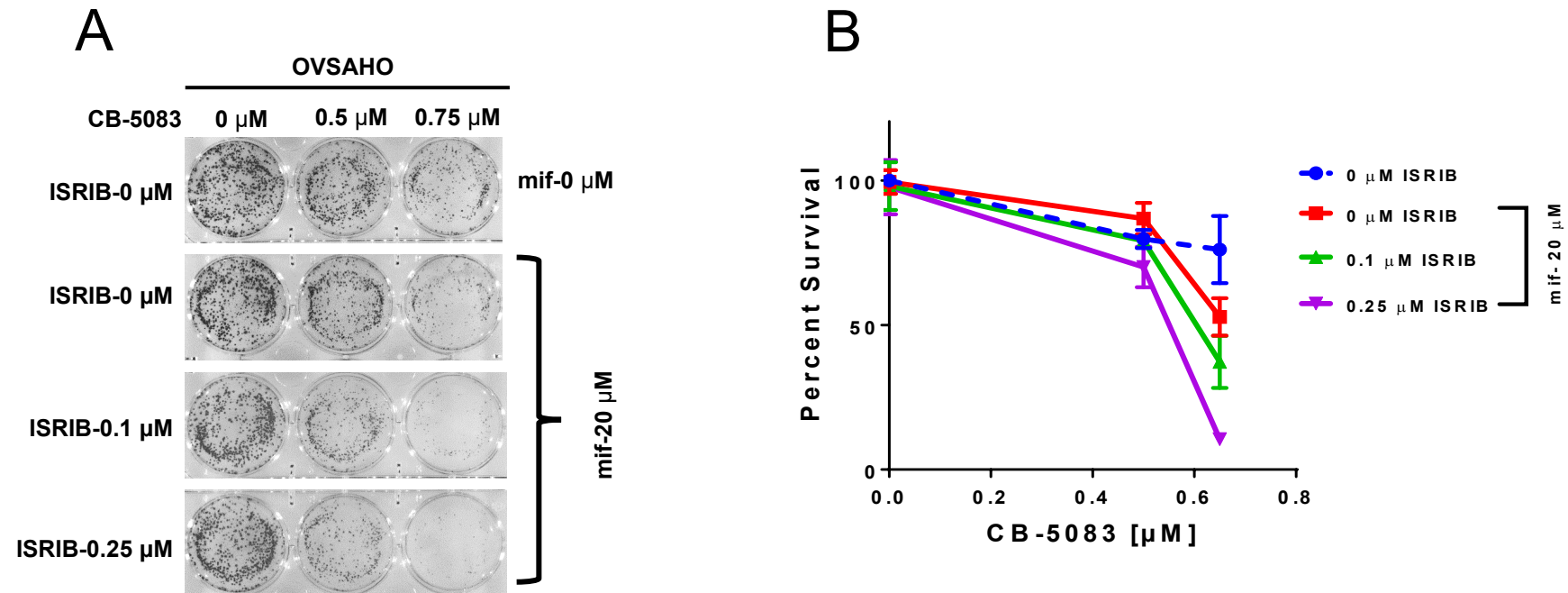
A: OVSAHO cells treated with indicated concentrations of DBeQ and mifepristone for 48 hours followed by 6-8 days of recovery in regular media. Percent survival was calculated based on the number of colonies from three biological replicates. P values were calculated using the two-tailed student's t-test. B: Western blot analysis of total (T) and cleaved (CI) caspase 3, full length (FL) and cleaved PARP1, and ER stress-related proteins at 12 hours and 24 hours after CB-5083 and/or mifepristone (mif). B. Immunoblot (IB) analysis of ubiquitinated (K48-linked) proteins following CB-5083 or mifepristone treatment in OVSAHO cells. C: RMG1 spheroids were treated with indicated concentrations of vehicle (DMSO), CB-5083 and mifepristone for 72 hours. Percent cell death was analyzed using 3D CellTiter-Glo Assay from three biological replicates.

Figure S4



A: RMG1 were treated with increasing doses of mifepristone ranging from 10  $\mu\text{M}$  to 100  $\mu\text{M}$  for 72 hours. Dose-response curves were generated using GraphPad Prism based on the four parameters nonlinear regression. The curves were constrained at the top (100%) and the bottom (>0%). Every point in the dose-response curve represents Mean  $\pm$  SEM taken from three technical replicates for all cell lines. B: RMG1 cells were incubated with increasing doses of IRE1 $\alpha$  inhibitor- STF-083010 (5  $\mu\text{M}$  – 80  $\mu\text{M}$ ) with 2  $\mu\text{M}$  of tunicamycin for 10 hours.

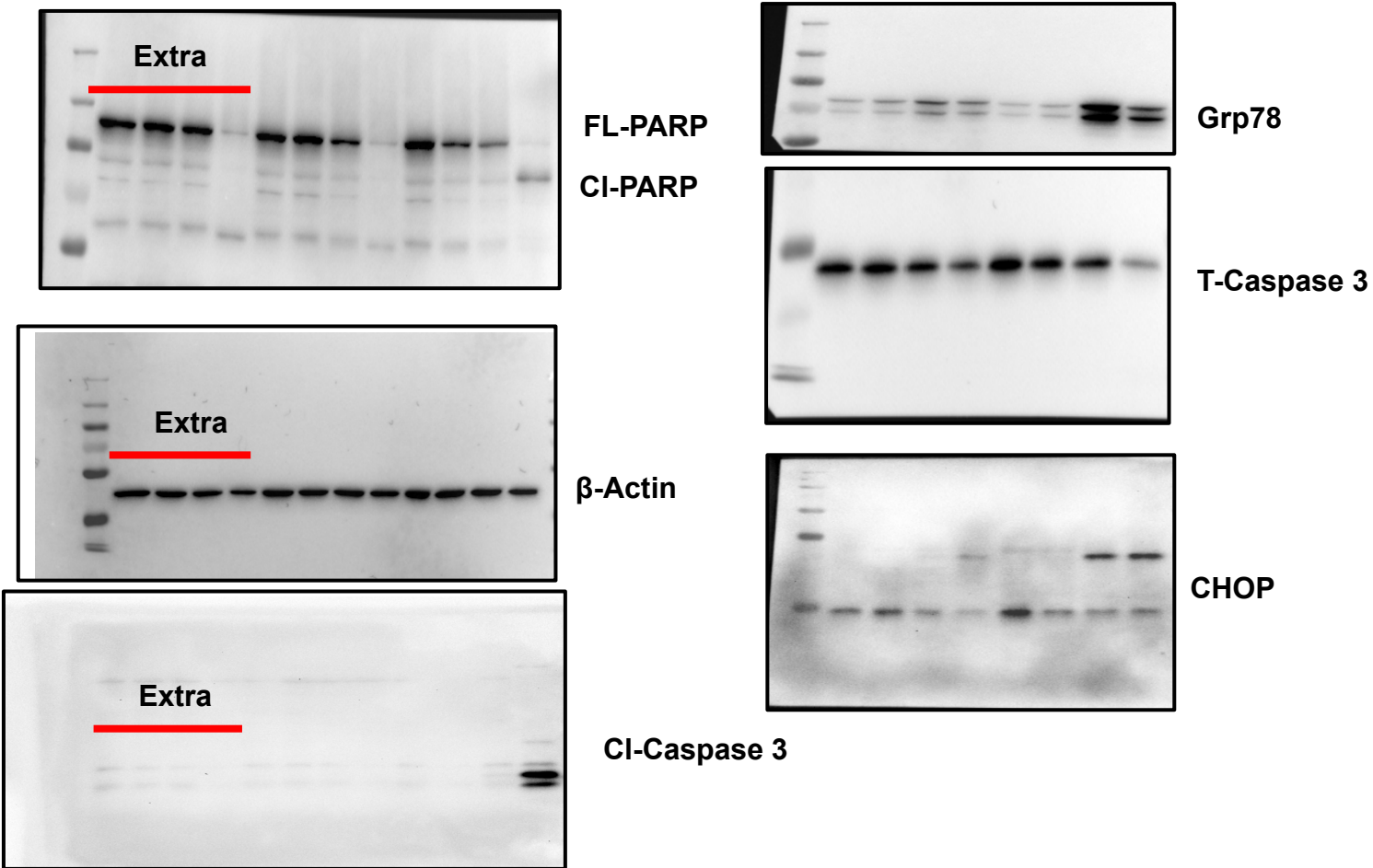
Figure S5



A: Clonogenic assay to assess the triple combination of CB-5083, ISRIB, and mifepristone in OVSAHO cells. B) Mean percent survival  $\pm$  SEM was calculated based on number of colonies from three biological replicates.

Figure S6 – Original western blot from Figure 3

A



B

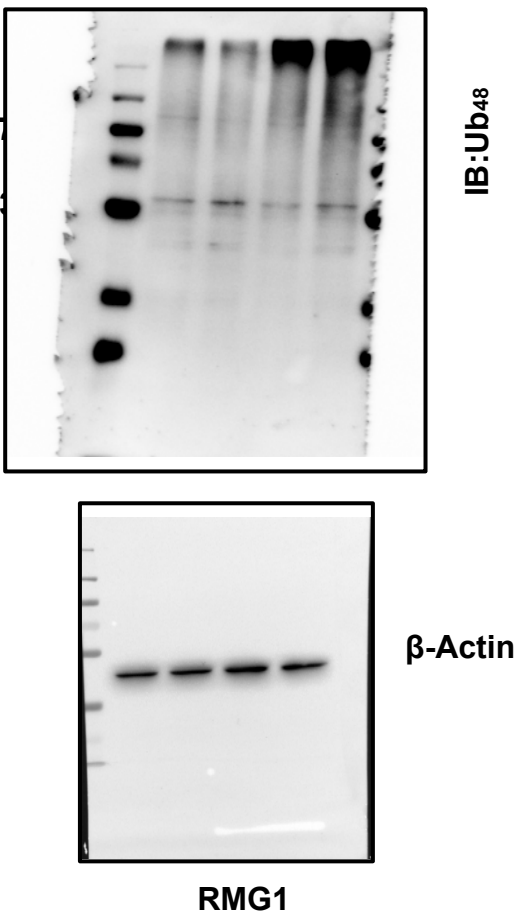


Figure S7 – Original western blot from Figure 4

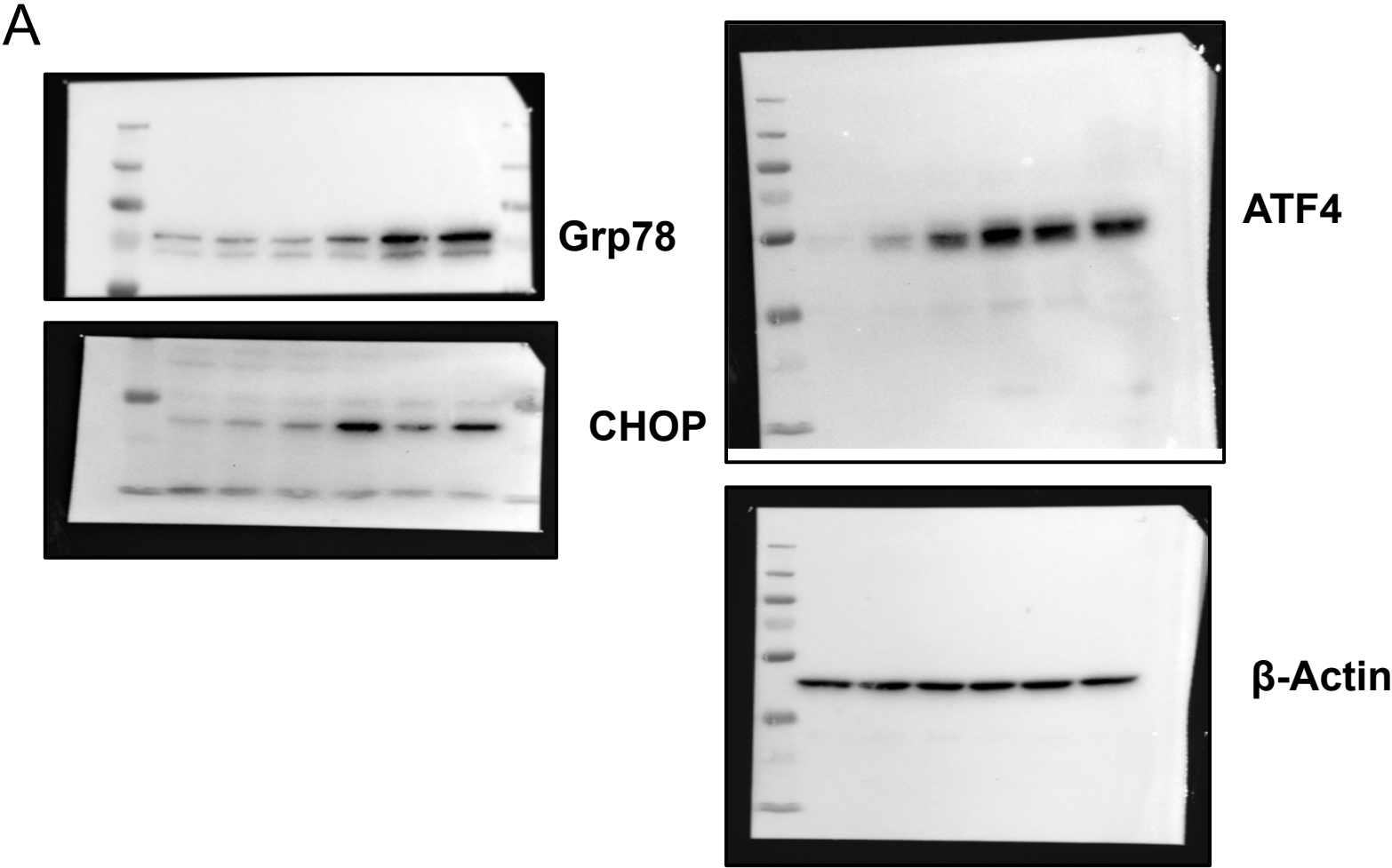
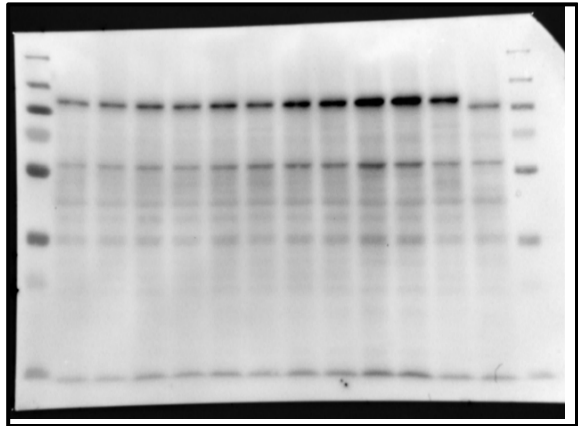
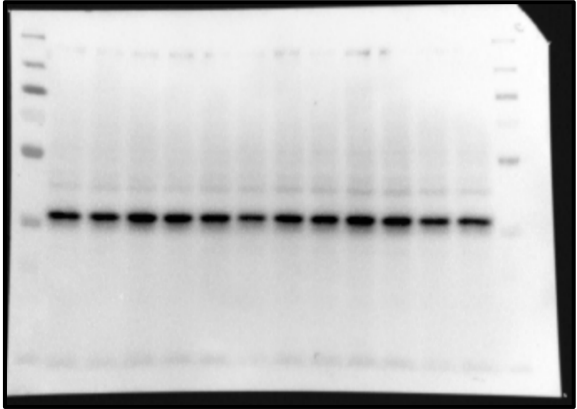




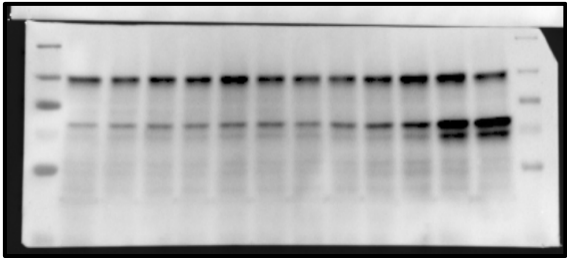
Figure S8 – Original western blot from Figure 5A



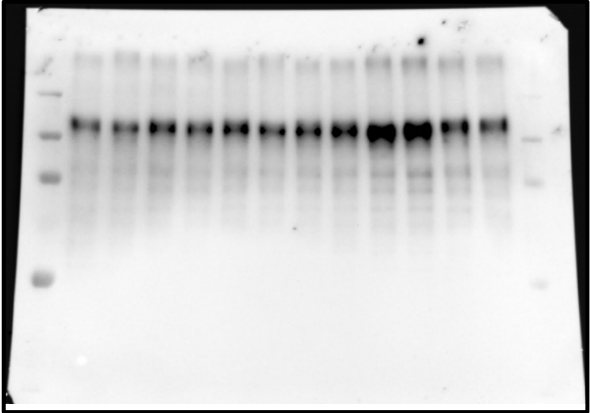
ATF6-FL



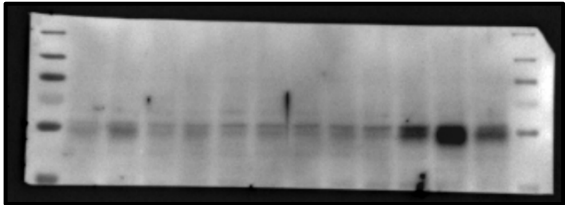
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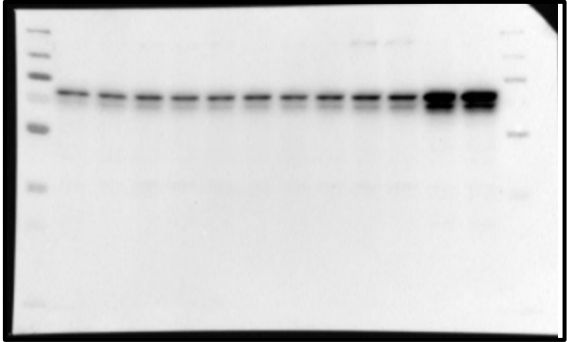
IRE1α



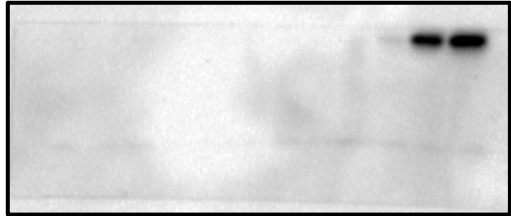
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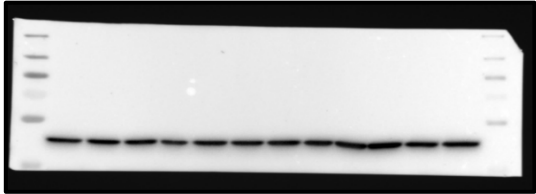
ATF4



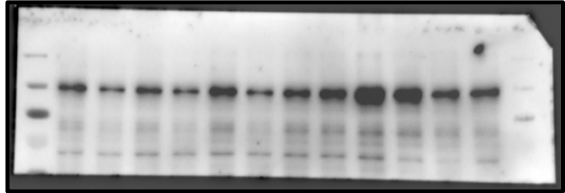
Grp78



CHOP

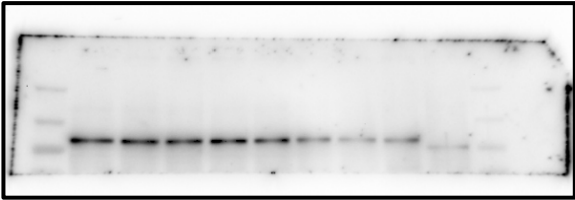


β-Actin

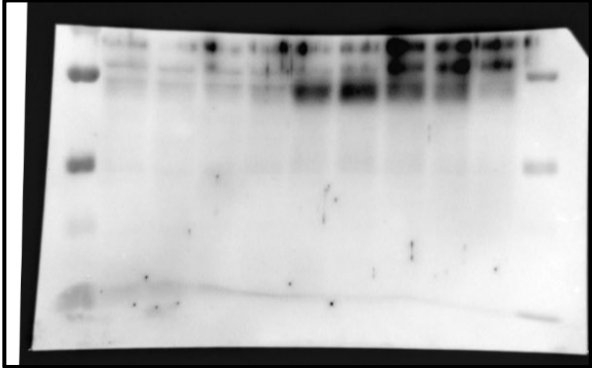


p-IRE1α

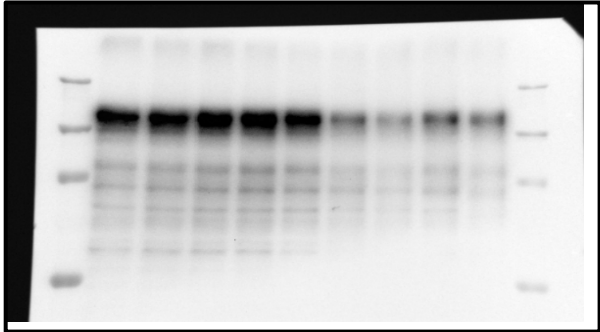
Figure S9 – Original western blot from Figure 5B



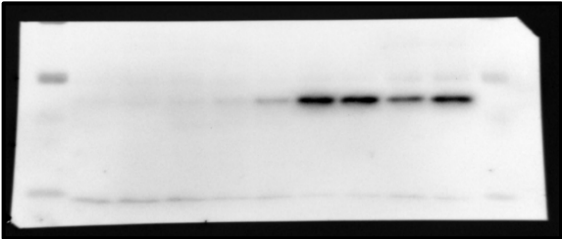
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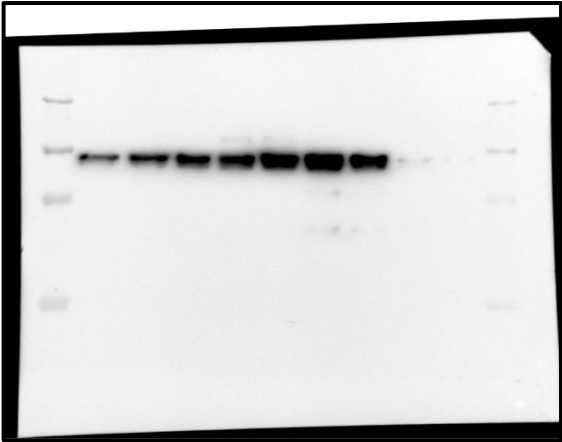
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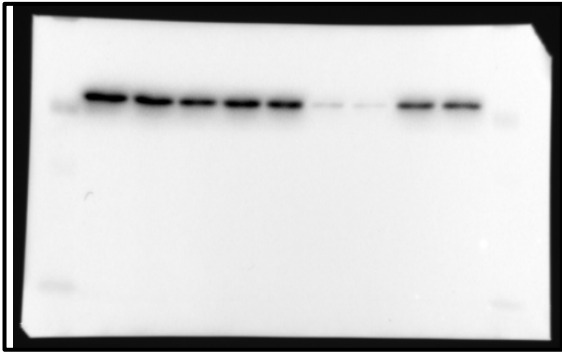
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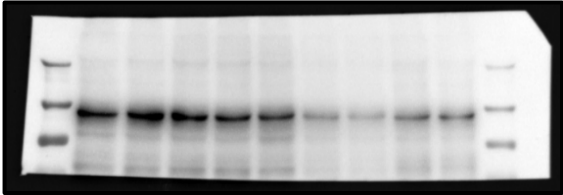
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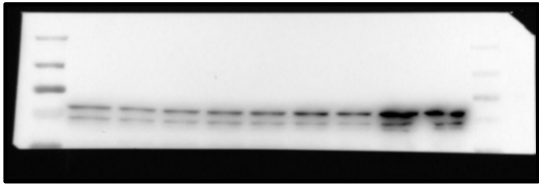
IRE1α



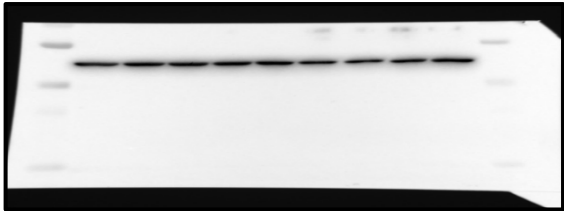
p-eIF2α



p-IRE1α



Grp78



β-Actin

Figure S10 – Original western blot from Figure 5C

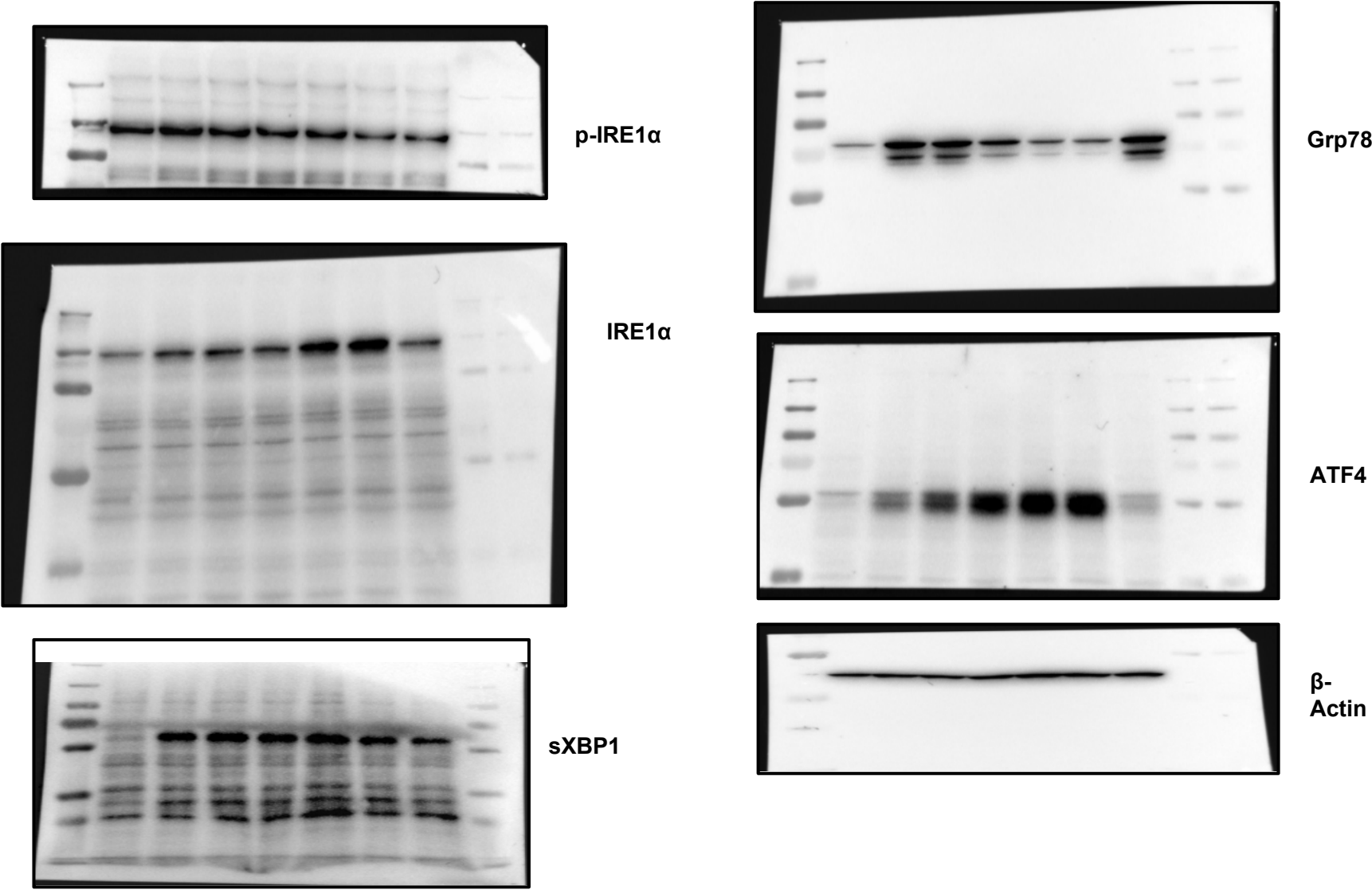
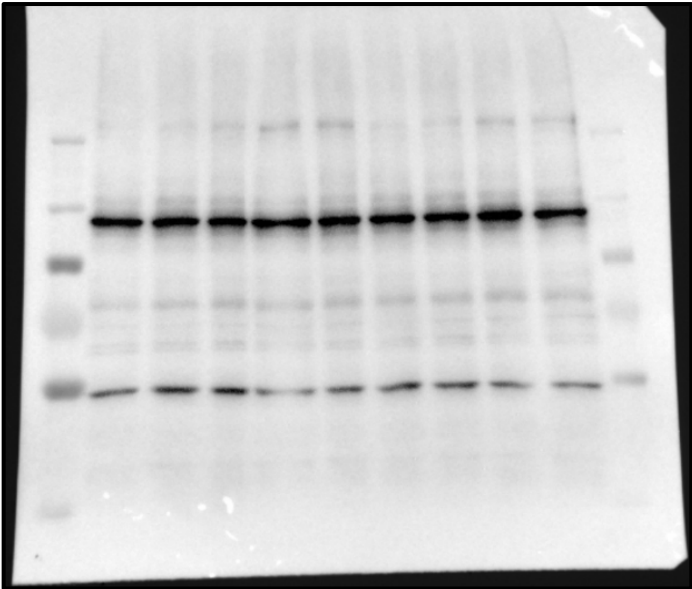
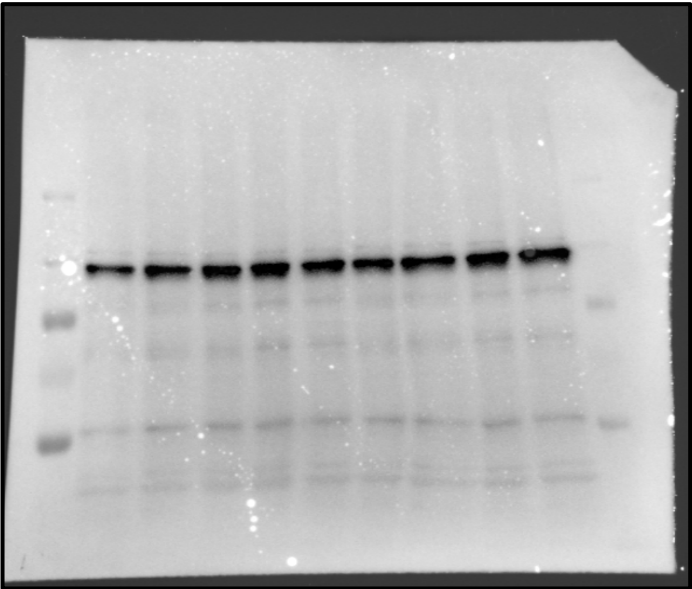


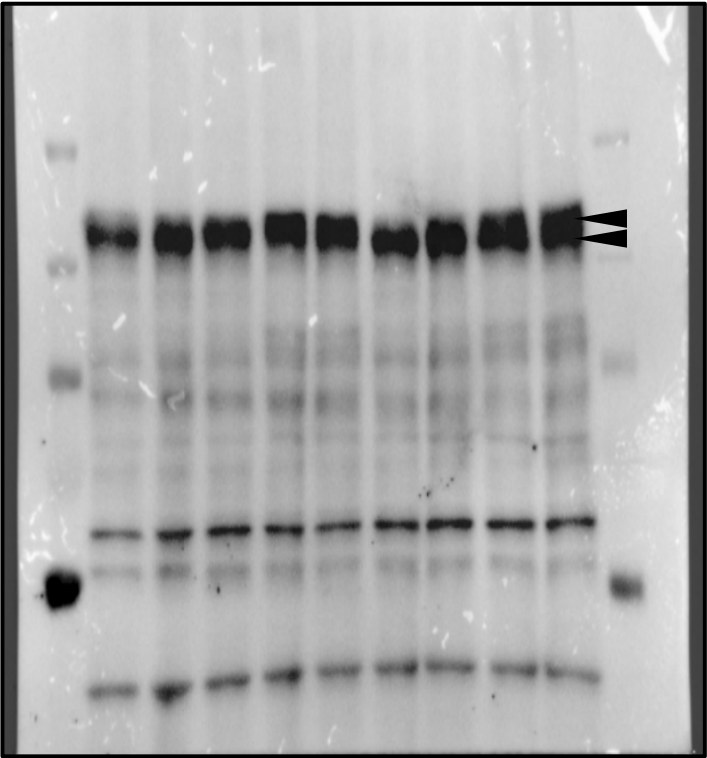
Figure S11 – Original western blot from Figure 5D



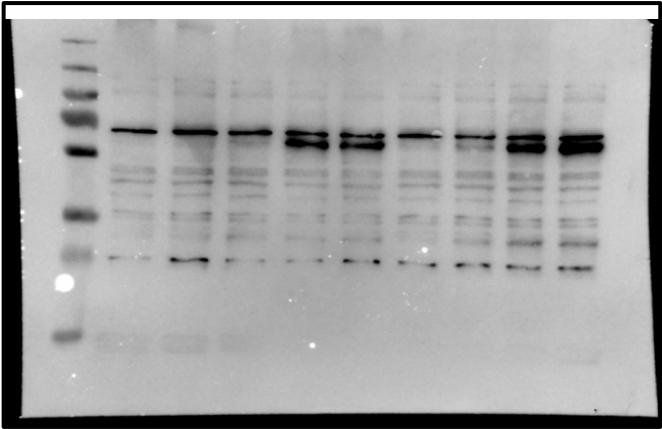
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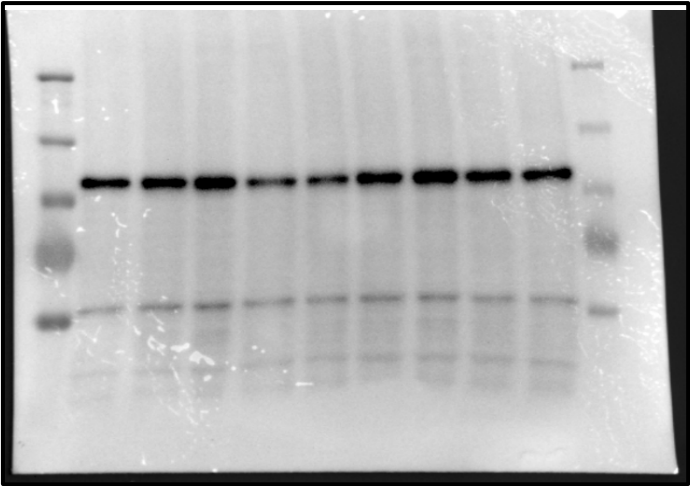
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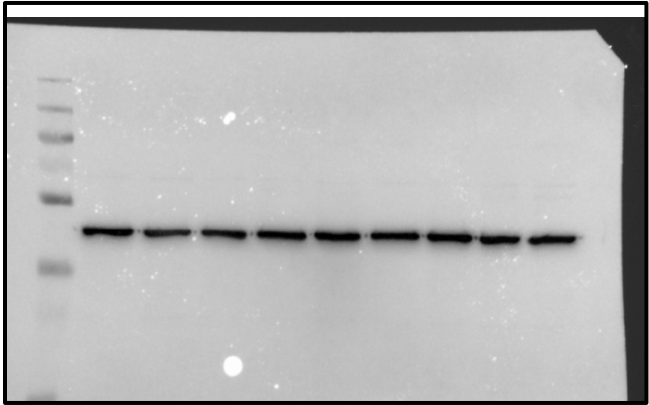
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PERK



s-XBP1



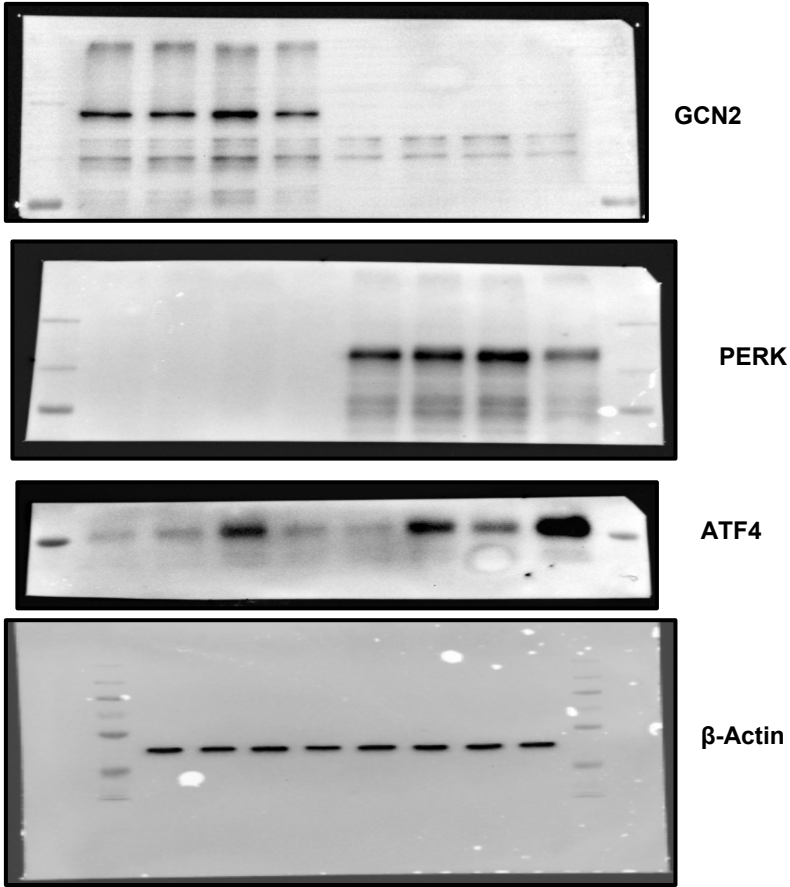
ATF6-FL



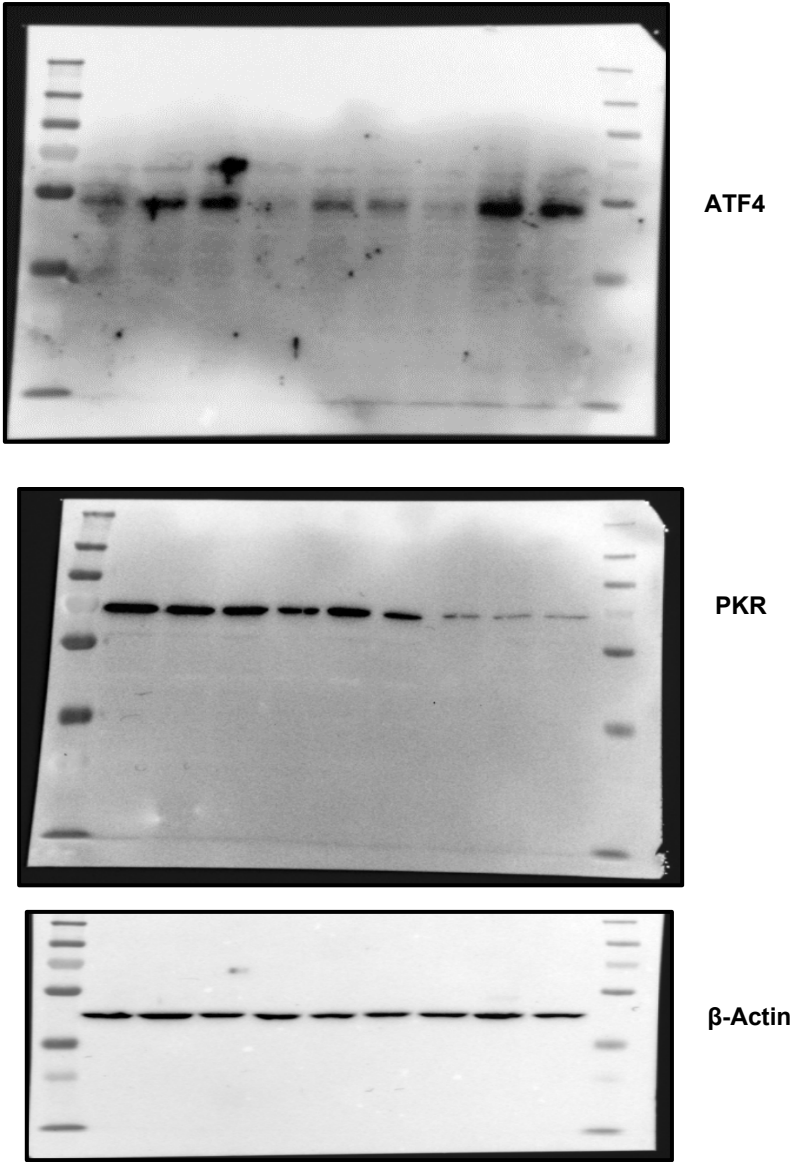
β-Actin

Figure S12 – Original western blot from Figure 6

A



B



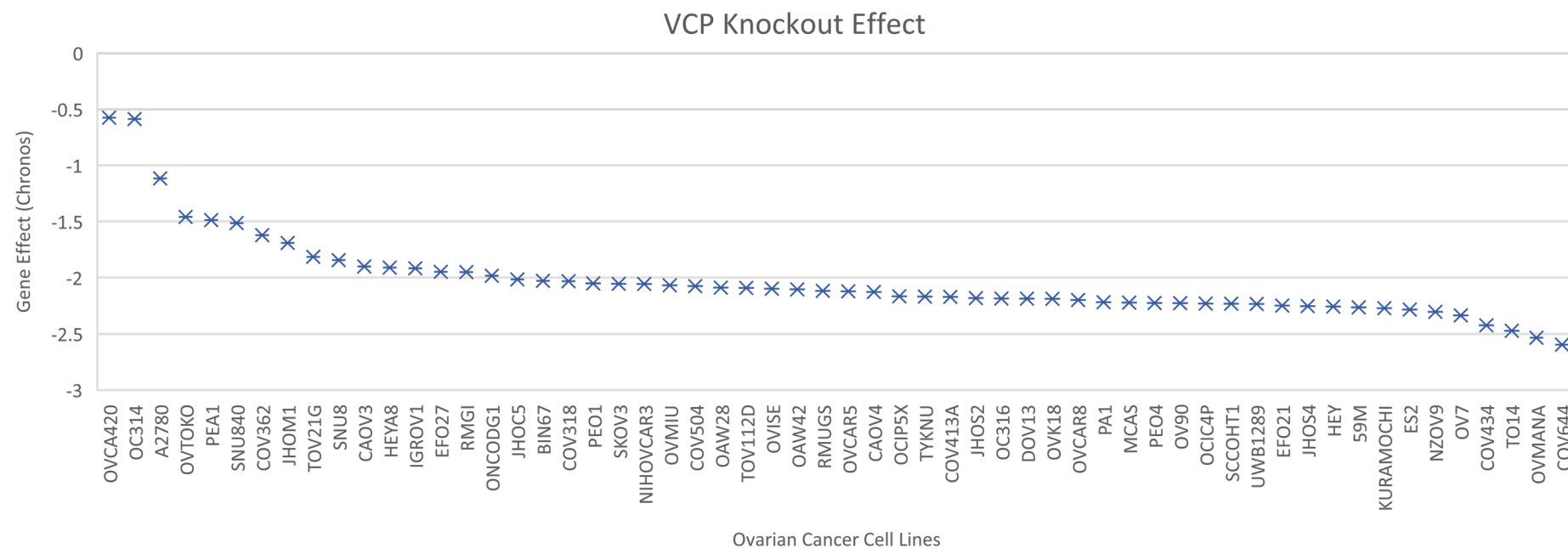


Figure S13. Effect of CRISPR/Cas9-mediated gene knockout of VCP in ovarian cancer cells. Negative gene effect indicates dependency. Gene effect < -1 suggest strong dependency on the gene. Data is downloaded from the DepMap project and replotted with Microsoft Excel. COV318, ONCODG1, OVCA8, and SNU8 are reported to have the CCNE1 amplification.

## Expression pattern of input genes in Ovarian serous cystadenocarcinoma (OV) ≡

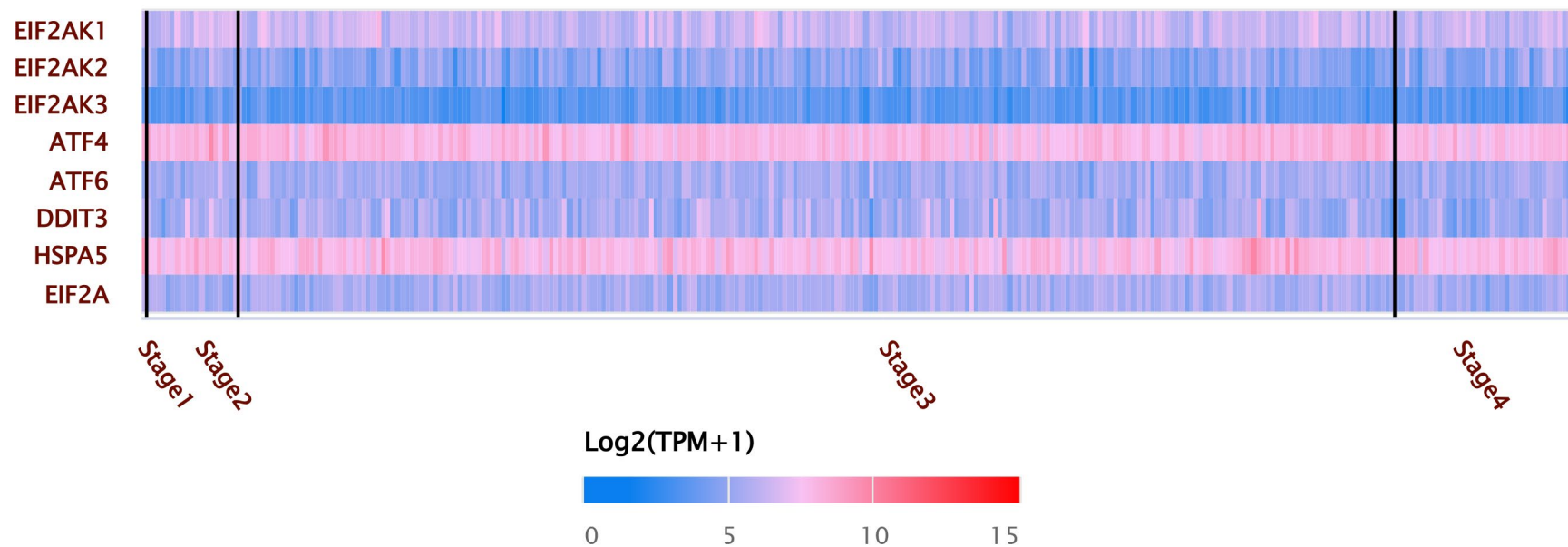


Figure S14 A: Analysis of the expression of select UPR-related genes in high-grade serous carcinomas from the TCGA dataset (from UALCAN).

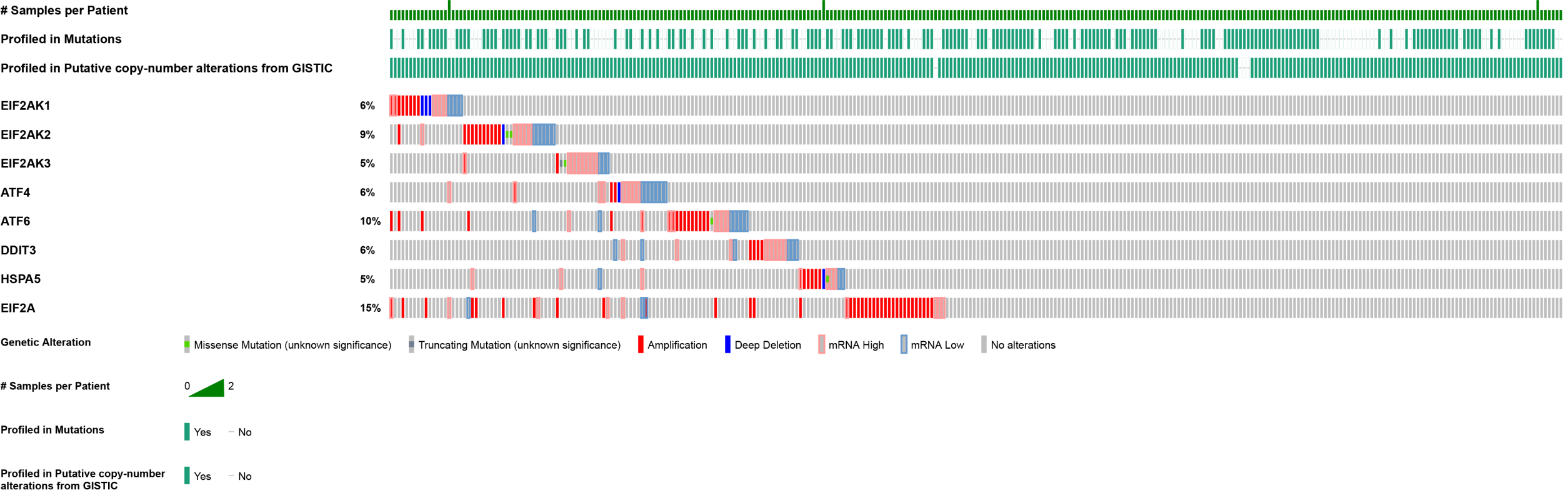
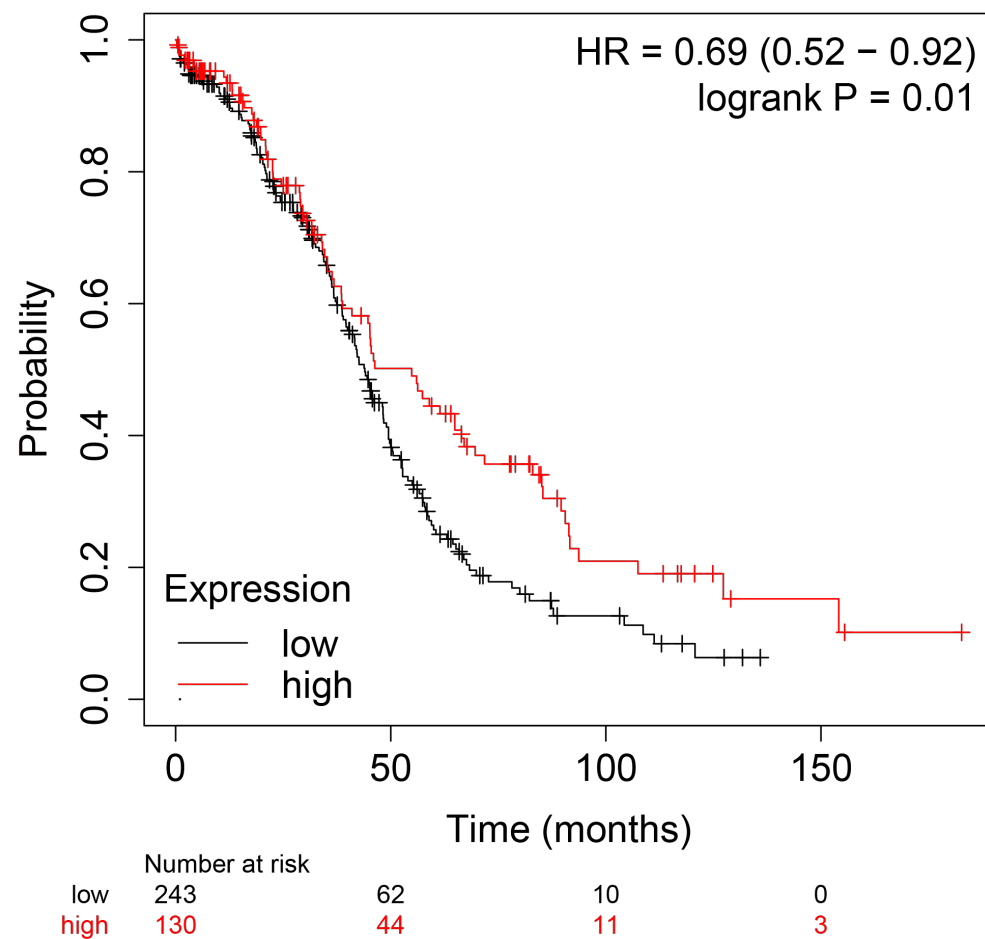


Figure S14 B. Analysis of genetic alterations in select UPR-related genes in high-grade serous carcinomas from the Cancer Genome Atlas dataset (from cbiportal).



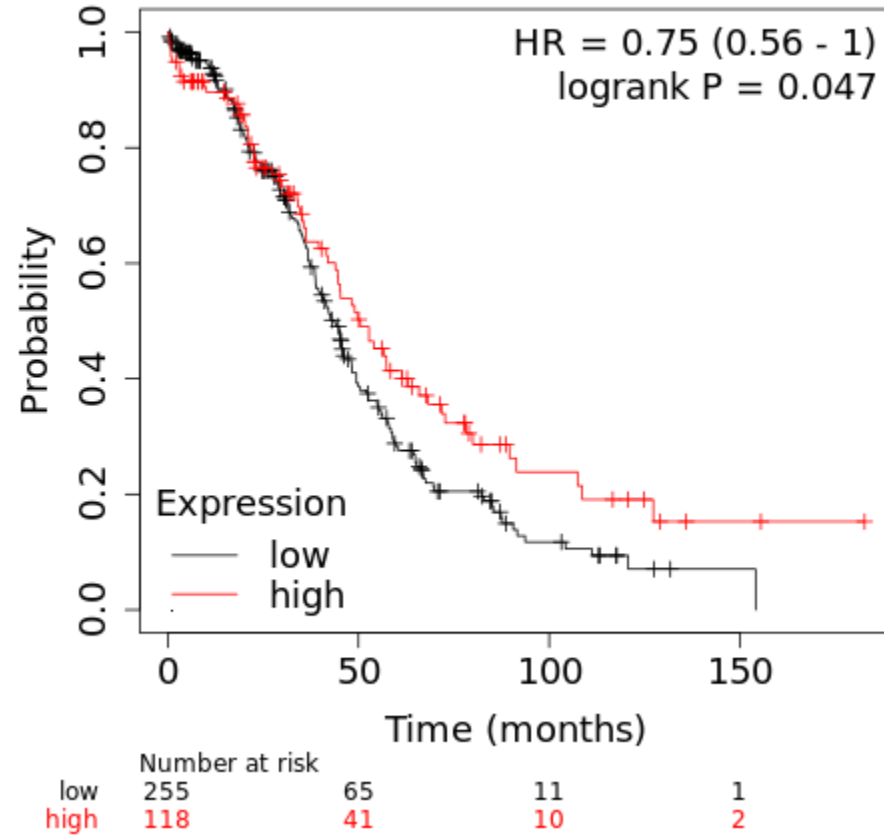
# EIF2AK1



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Figure S14C

## EIF2AK2



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Figure S14C

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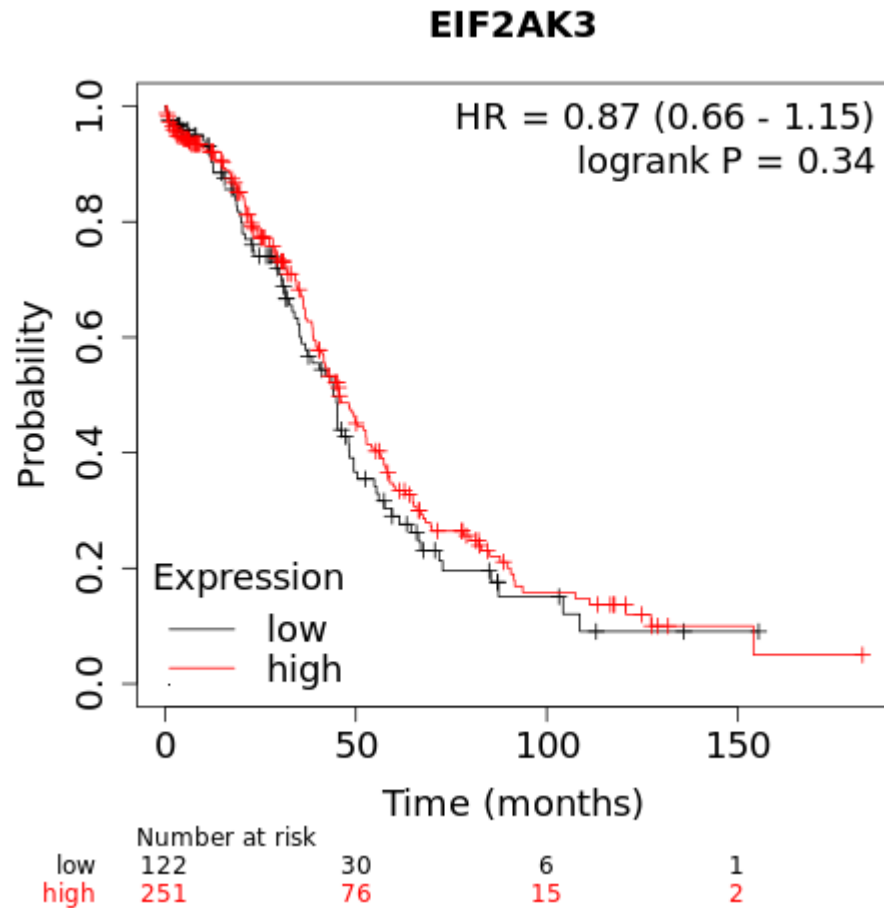
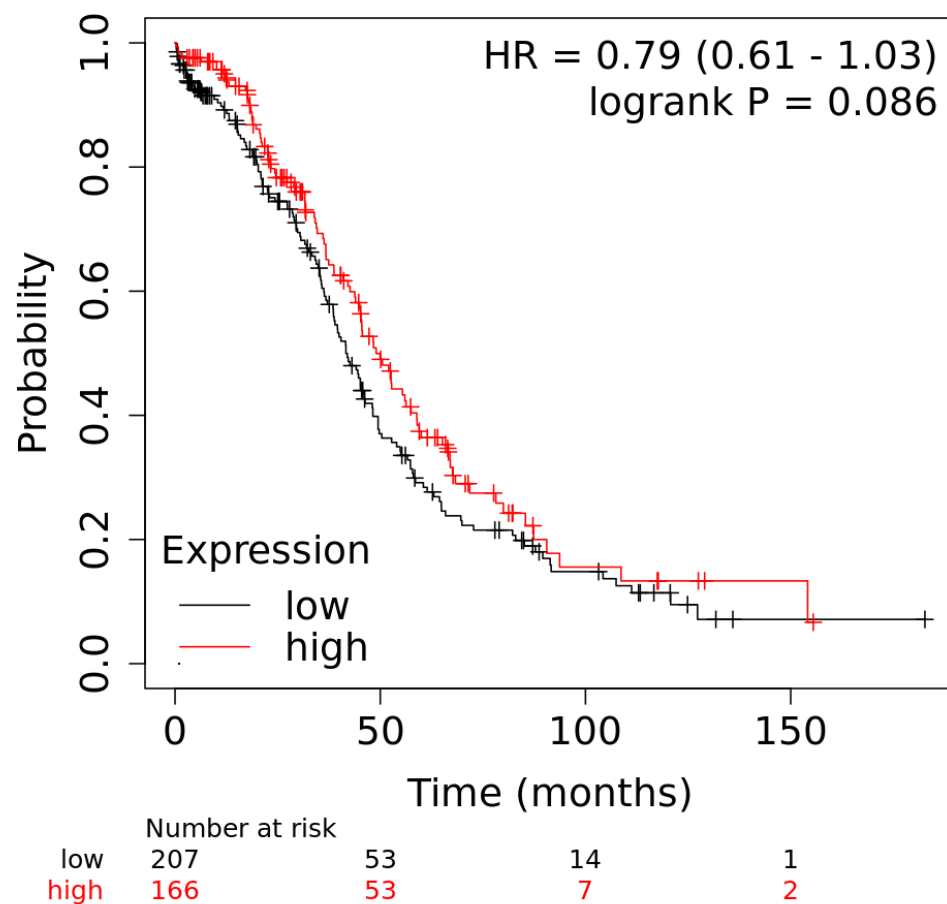


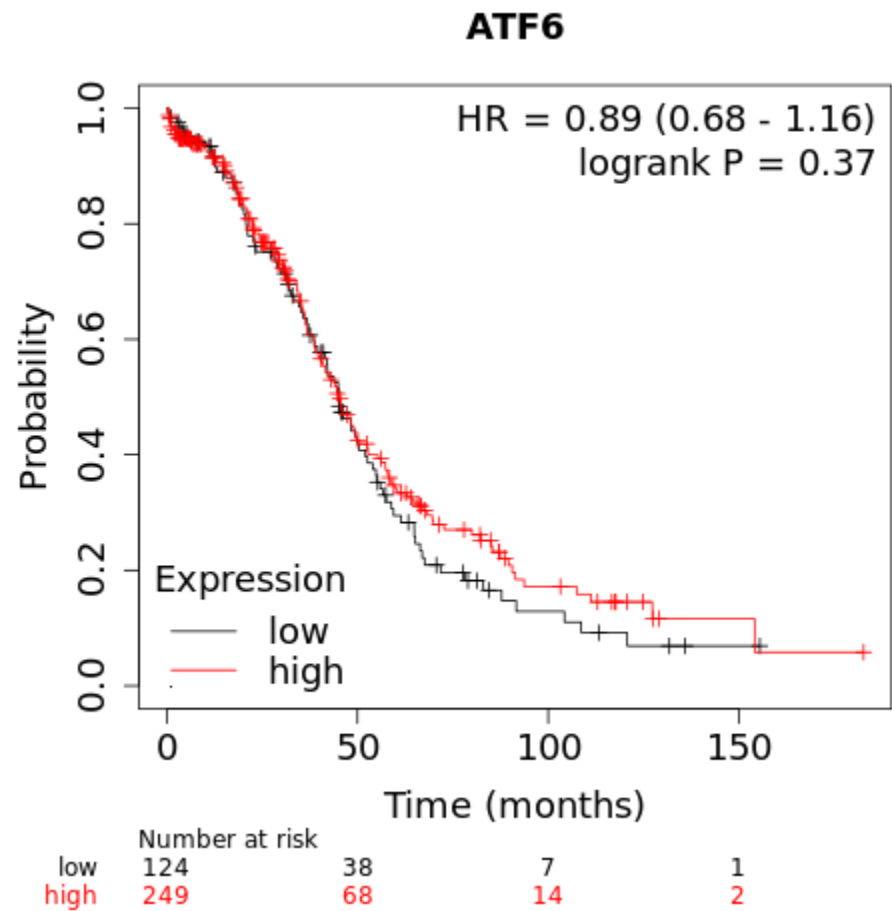
Figure S14C

# ATF4



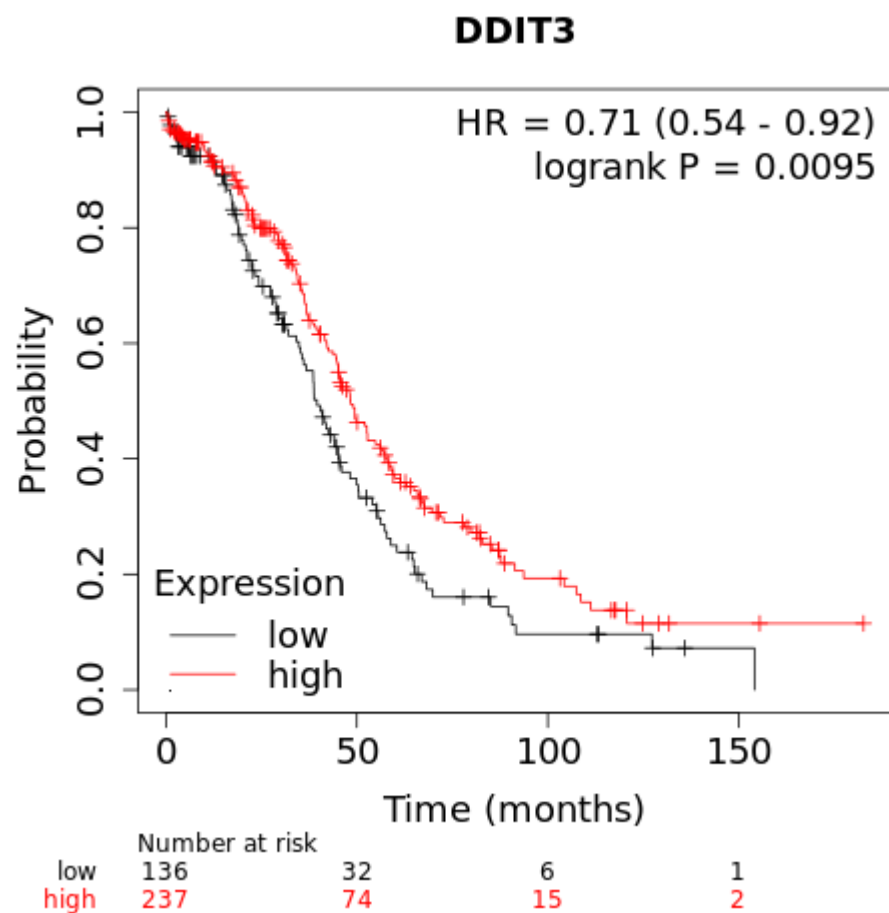
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Figure S14C



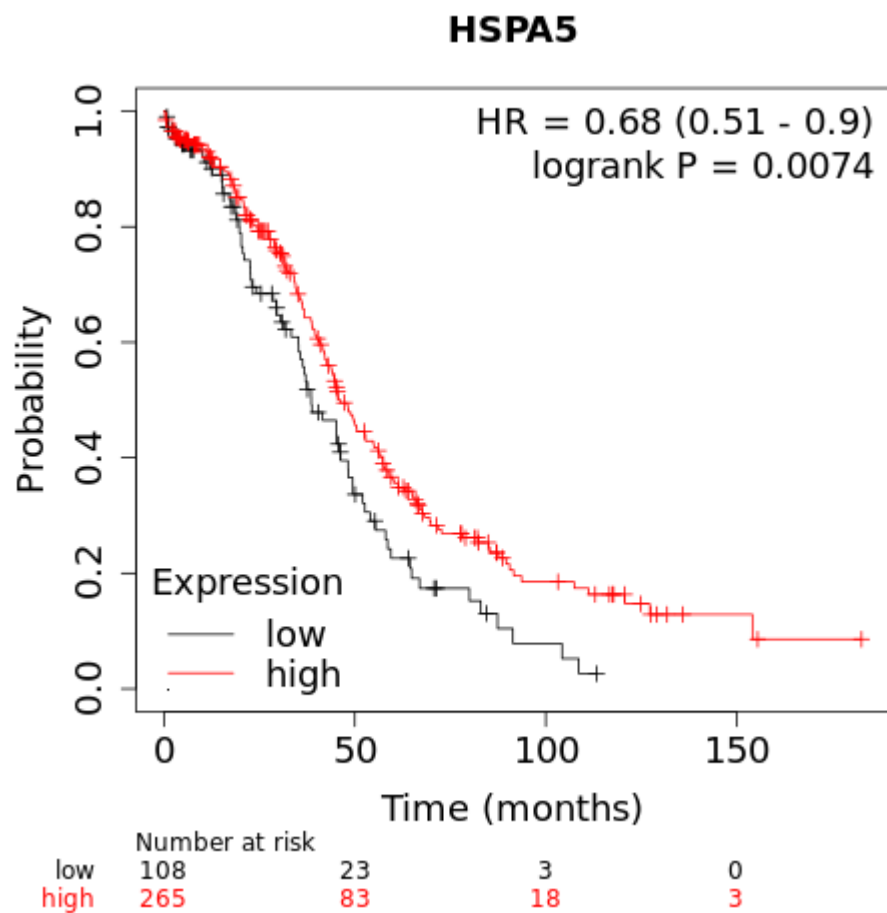
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Figure S14C



[https://kmplot.com/analysis/index.php?p=view&pa\\_id=15382068&show=bZFNboQwDIXvwrqqNltuehnLBGewCHfKO8y0o969gUULiO33np\\_\\_Xp3hQoAGzjF2n13EZNS9dTbKA2YaGHOjrvUfcp7xeYI9kT1Q5-sEkAhWdeEF07kQw9QKB1CKpJQDnSNKEofEmcBHDIMms2a5HWSnp0MvabhWjL\\_X2Nv7x0EL1VxmmJFzW9\\_TdevShq4ERYydZbuGFOX76McJ6U552Nt68RafKK7GolLCSGHa9fhjgCk98Mt2WjWCBZWxTwRRFIKMor63BFEFK9TOvj3pwFdsks8bheqy\\_Xn7wc8v](https://kmplot.com/analysis/index.php?p=view&pa_id=15382068&show=bZFNboQwDIXvwrqqNltuehnLBGewCHfKO8y0o969gUULiO33np__Xp3hQoAGzjF2n13EZNS9dTbKA2YaGHOjrvUfcp7xeYI9kT1Q5-sEkAhWdeEF07kQw9QKB1CKpJQDnSNKEofEmcBHDIMms2a5HWSnp0MvabhWjL_X2Nv7x0EL1VxmmJFzW9_TdevShq4ERYydZbuGFOX76McJ6U552Nt68RafKK7GolLCSGHa9fhjgCk98Mt2WjWCBZWxTwRRFIKMor63BFEFK9TOvj3pwFdsks8bheqy_Xn7wc8v)

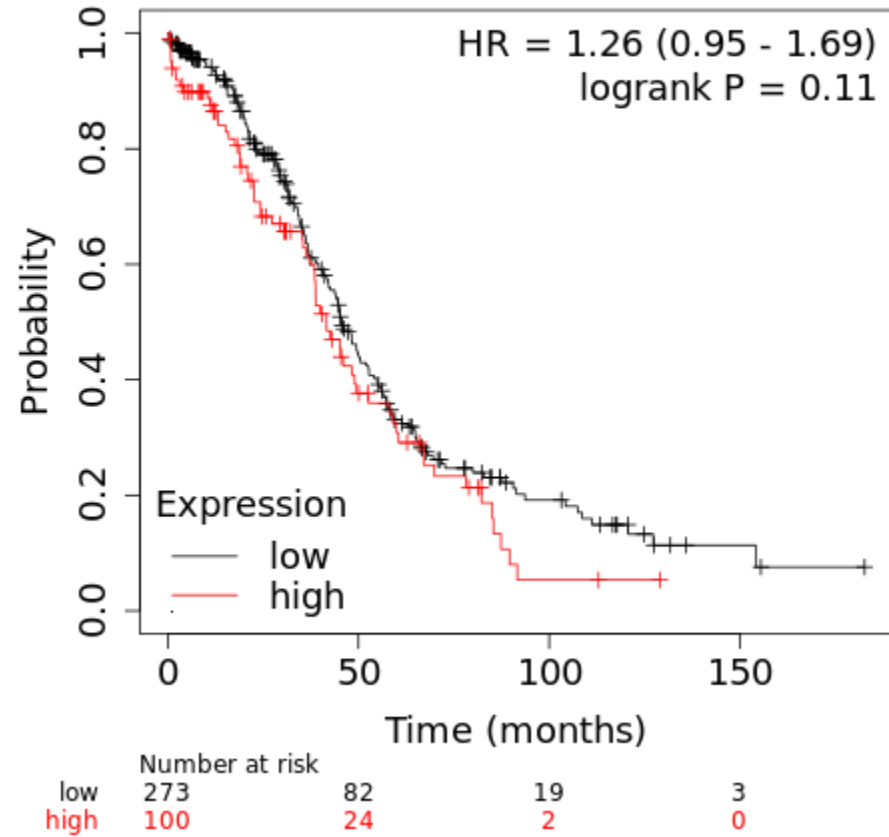
Figure S14C



[https://kmplot.com/analysis/index.php?p=view&pa\\_id=15382069&show=bZFNboQwDIXvwrqqNltuehnLBGewCHFkO8y0o969gUULiO33np\\_\\_Xp3hQoAGzjF2n13EZNS9dTbKA2YaGHOjrvUfcp7xeYl9kT1Q5-sEkAhWdeEF07kQw9QKB1CKpJQDnSNKEofEmcBHDIMms2a5HWSnp0MvabhWjL\\_X2Nv7x0EL1VxmmJFzW9\\_TdevShq4ERYydZbuGFOX76McJ6U552Nt68RafKK7GolLCSGHa9fhjgCk98Mt2WjWCBZWxTwRRFIKMor63BFEFK9TOvj3pwFdsk8bheqy\\_Xn7wc8v](https://kmplot.com/analysis/index.php?p=view&pa_id=15382069&show=bZFNboQwDIXvwrqqNltuehnLBGewCHFkO8y0o969gUULiO33np__Xp3hQoAGzjF2n13EZNS9dTbKA2YaGHOjrvUfcp7xeYl9kT1Q5-sEkAhWdeEF07kQw9QKB1CKpJQDnSNKEofEmcBHDIMms2a5HWSnp0MvabhWjL_X2Nv7x0EL1VxmmJFzW9_TdevShq4ERYydZbuGFOX76McJ6U552Nt68RafKK7GolLCSGHa9fhjgCk98Mt2WjWCBZWxTwRRFIKMor63BFEFK9TOvj3pwFdsk8bheqy_Xn7wc8v)

Figure S14C

## EIF2A



[https://kmplot.com/analysis/index.php?p=view&pa\\_id=15382070&show=bZFNboQwDIXvwrqqNltuehnLBGewCHFkO8y0o969gUULiO33np\\_\\_Xp3hQoAGzjF2n13EZNS9dTbKA2YaGHOjrvUfcp7xeYI9kT1Q5-sEkAhWdeEF07kQw9QKB1CKpJQDnSNKEofEmcBHDIMms2a5HWSnp0MvabhWjL\\_X2Nv7x0EL1VxmmJFzW9\\_TdevShq4ERYydZbuGFOX76McJ6U552Nt68RafKK7GolLCSGHa9fhjgCk98Mt2WjWC BZWxTwRRFIKMor63BFEFK9TOvj3pwFdsk8bheqy\\_Xn7wc8v](https://kmplot.com/analysis/index.php?p=view&pa_id=15382070&show=bZFNboQwDIXvwrqqNltuehnLBGewCHFkO8y0o969gUULiO33np__Xp3hQoAGzjF2n13EZNS9dTbKA2YaGHOjrvUfcp7xeYI9kT1Q5-sEkAhWdeEF07kQw9QKB1CKpJQDnSNKEofEmcBHDIMms2a5HWSnp0MvabhWjL_X2Nv7x0EL1VxmmJFzW9_TdevShq4ERYydZbuGFOX76McJ6U552Nt68RafKK7GolLCSGHa9fhjgCk98Mt2WjWC BZWxTwRRFIKMor63BFEFK9TOvj3pwFdsk8bheqy_Xn7wc8v)

Figure S14C



Figure S14 C. Overall survival analysis of select genes in high-grade serous carcinomas obtained from the Kaplan-Meier Plotter using the RNAseq dataset from the Pancancer survival analysis. Auto select best cutoff option is used to dichotomize into low and high expression groups. Each cutoff value for each plot can be viewed in permanent weblink provided next to the plot.