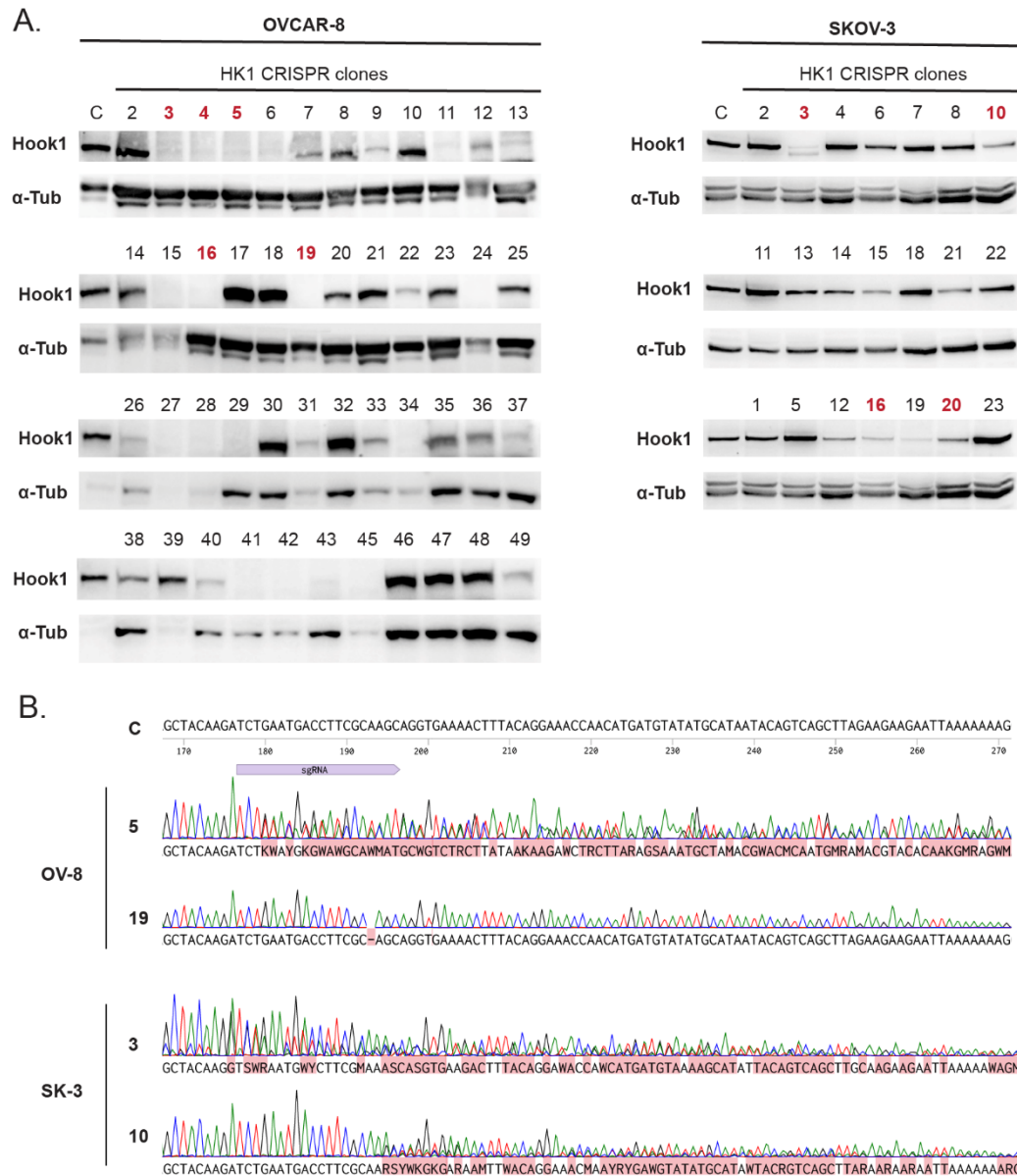
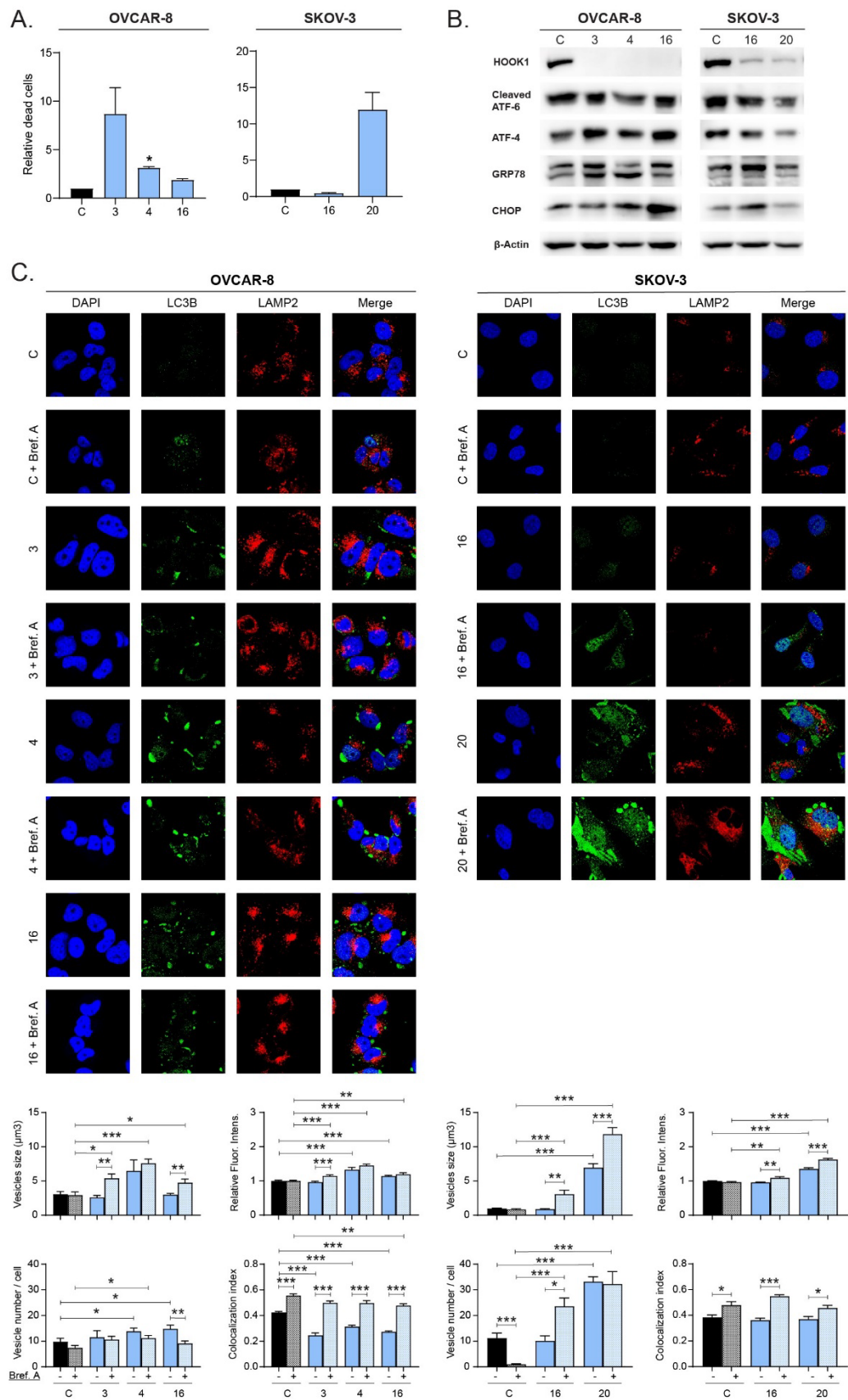


Supplementary Figures



Supplementary Figure 1. Generation and validation of HOOK1 CRISPRs in ovarian cancer cell lines. (A) Protein levels of HOOK1 after CRISPR–Cas9 system infection in ovarian cancer cell lines. The selected clones are highlighted in red. **(B)** DNA sequencing of the sgRNA region of clones 5 and 19 of OVCAR-8 CRISPR and clones 3 and 10 of SKOV-3 CRISPR compared to the original sequence.



Supplementary Figure 2. Analysis of cell death, ER stress and autophagy in different HOOK1 CRISPR clones in OVCAR-8 and SKOV-3 cell lines. (A) Quantification by flow cytometry of the relative number of dead cells when Hook1 is downregulated. **(B)** Protein levels of UPR-associated proteins in Hook1-downregulated cells. **(C)** Immunofluorescence staining of LC3B (green) and LAMP2 (red) proteins in Hook1-downregulated cells treated with brefeldin A. DAPI (blue) was used as a nuclear stain, and the merge of the 3 markers is shown. The autophagosome size, number and relative fluorescence intensity, as well as the colocalization index of autophagosomes-lysosomes, were measured using ImageJ software. The statistical analysis was performed using Student's t test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

A.

OVCAR-8

Process	Go term	Associated genes
Protein folding	Protein folding in endoplasmic reticulum	HSPA5 HSP90B1 PDIA3 CALR
	ATF6-mediated unfolded protein response	HSPA5 HSP90B1 CALR
	ER unfolded protein response/ Cellular response to unfolded protein	HSPA5 PDIA6 LMNA HSP90B1 CALR GFPT1
	Cellular response to topologically incorrect protein	HSPA5 UGGT1 PDIA6 LMNA HSP90B1 CALR GFPT1
	Response to unfolded protein	HSPA5 PDIA6 MANF LMNA HSP90B1 CALR GFPT1
	Response to topologically incorrect protein	HSPA5 UGGT1 PDIA6 MANF LMNA HSP90B1 CALR GFPT1
	Response to ER stress	HSPA5 UGGT1 PDIA6 MANF PDIA4 LMNA HSP90B1 PDIA3 CALR GFPT1
Metabolic process	Protein folding	HSPA5 UGGT1 PDIA6 PDIA4 HSP90B1 PDIA3 CALR
	UDP-N-acetylglucosamine/ amino sugar biosynthetic process	PGM3 AMDHD2 GFPT1
	L-ascorbic acid/ lactone metabolic process	SLC2A3 SLC2A1 AKR1A1
	Nucleotide-sugar metabolic process	PGM3 UGGT1 AMDHD2 GFPT1
Cytoskeleton organization	Negative regulation of supramolecular fiber organization	CAPG LIMA1 ARHGEF2 LDLR HIP1R GSN
	Actin cytoskeleton organization/ Actin filament-based process	CAPG LIMA1 ACTN1 ICAM1 ARHGEF2 PALLO HIP1R RAB13 GSN HSP90B1 CALR
	Cytoskeleton organization	CAPG LIMA1 ACTN1 ICAM1 CNTNAP1 ARHGEF2 CENPF PALLO CLIP1 HIP1R RAB13 GSN LMNA HSP90B1 CALR
Others	Response to organonitrogen compound	CD9 HSPA5 CYBA ICAM1 ARHGEF2 SLC2A1 LDLR UGGT1 USO1 RAB13 GSN RGS10 HSP90B1
	Response to oxygen-containing compound	CD9 HSPA5 CYBA ICAM1 MTAP ARHGEF2 SLC2A1 AKR1A1 LDLR USO1 PFKL RAB13 GSN RGS10 HSP90B1 CALR SMYD3

SKOV-3

Process	Go term	Associated genes
Migration	Mesenchyme migration	ACTA2 ACTA1 ACTC1 ACTG2
	Tissue migration	ANLN HSPB1 ACTA2 ANXA1 ACTA1 ACTC1 ACTG2 ITGB3
Protein folding	Protein folding in endoplasmic reticulum	HSPA5 HSP90B1 P4HB
Cytoskeleton organization	Neg. Reg. of actin filament depolymerization	LIMA1 SPTBN1 GSN SPTAN1
	Actin filament-based movement	VIM ACTA1 GSN MYO1E ACTC1 FLNA
	Actin filament organization	LIMA1 PDLM1 SPTBN1 ACTA1 GSN MYO1E ACTC1 HSP90B1 FLNA SPTAN1 CD2AP
	Actin cytoskeleton organization	ANLN LIMA1 PDLM1 SPTBN1 ANXA1 SDCBP ACTA1 GSN MYO1E ACTC1 HSP90B1 FLNA SPTAN1 CD2AP
	Actin filament-based process	ANLN VIM LIMA1 PDLM1 SPTBN1 ANXA1 SDCBP ACTA1 GSN MYO1E ACTC1 HSP90B1 FLNA SPTAN1 CD2AP
	Supramolecular fiber organization	VIM LIMA1 PDLM1 SPTBN1 ACTA1 GSN MYO1E ACTC1 HSP90B1 P4HB FLNA SPTAN1 CD2AP
	Cytoskeleton organization	ANLN VIM LIMA1 PDLM1 CDC6 GAPDH SPTBN1 CDC20 CCNB1 ANXA1 SDCBP ACTA1 GSN MYO1E ACTC1 HSP90B1 FLNA SPTAN1 CD2AP
Secretion	Exocytosis	VCL HUWE1 CPED1 ANXA1 SDCBP GSN TAGLN2 PGAM1 FLNA IGF2R SPTAN1 ITGB3
	Secretion by cell	VCL HUWE1 CPED1 OSBP ANXA1 SDCBP GSN PDIA4 TAGLN2 PGAM1 FLNA IGF2R SPTAN1 CD2AP ITGB3
	Muscle system process	VIM VCL ENO1 ACTA2 ACTA1 GSN ACTC1 ACTG2 FLNA
Others	Neg. reg. of cellular component organization	VIM HSPA5 LIMA1 HUWE1 SPTBN1 CDC20 TOP2A CCNB1 SDCBP GSN FLNA SPTAN1 ITGB3
	Reg. of organelle organization	LIMA1 ENO1 HUWE1 OSBP SPTBN1 CDC20 TOP2A CCNB1 ANXA1 SDCBP GSN FLNA SPTAN1 CD2AP

B.

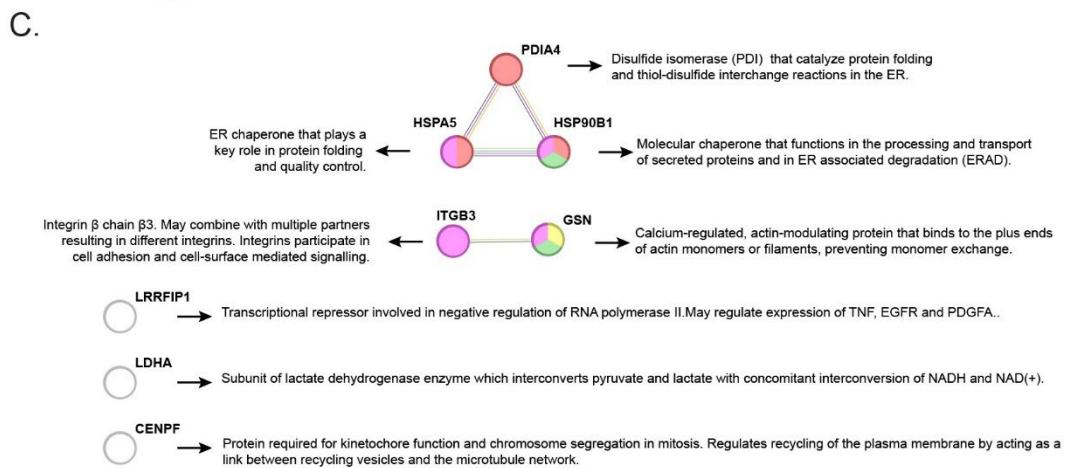
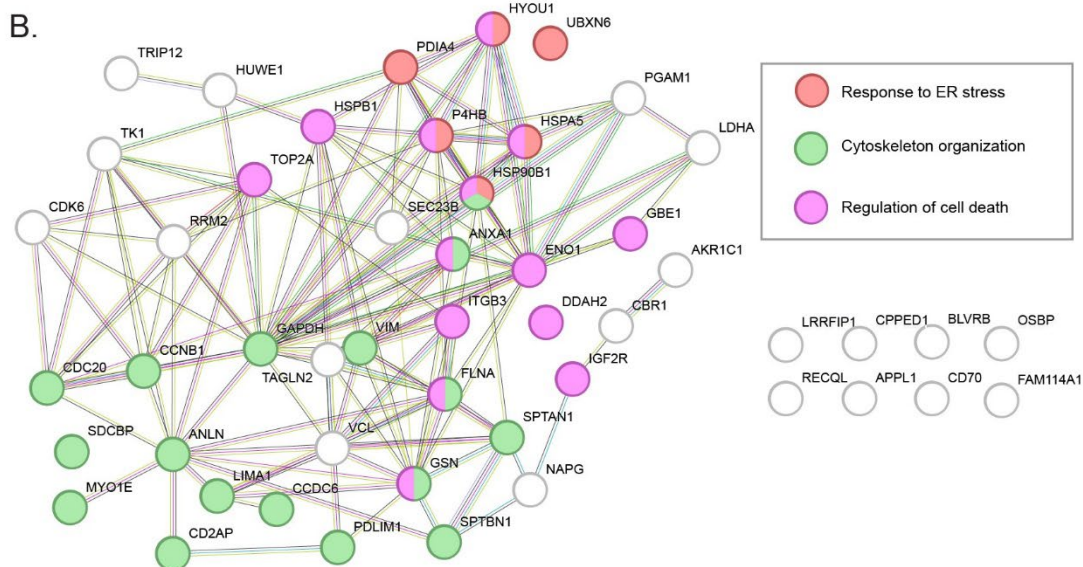
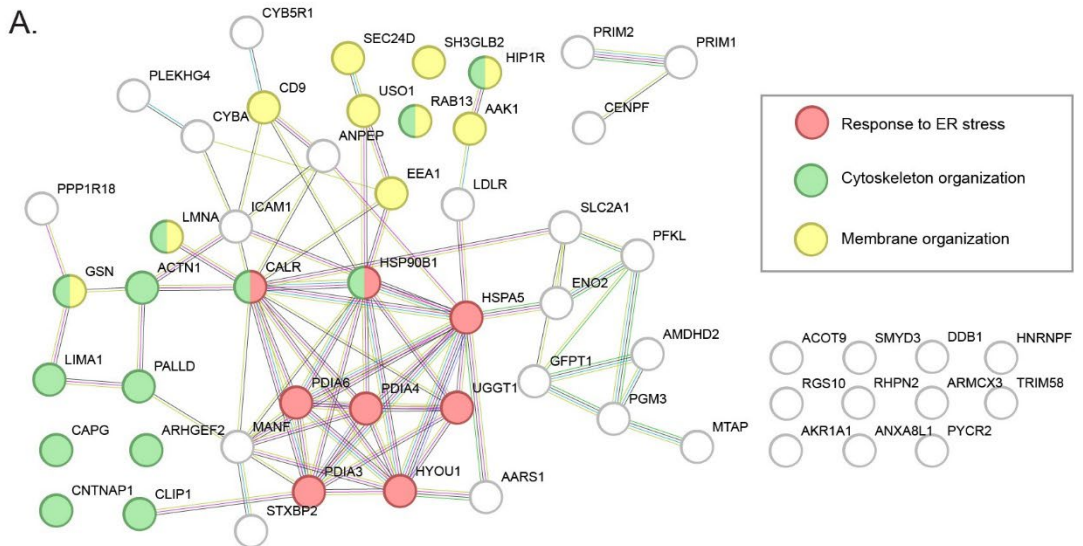
OVCAR-8

Process	Go term	Associated genes
Regulation of RNA	RNA splicing	DDX5 HNRNPH3 GEMINS U2AF2 GEMIN4 HNRNPH1 PTBP2 PUF60
	ncRNA metabolic process	GLTSCR2 DDX21 PRKDC GEMIN4 RRS1 GTPBP4 LARS TAR5 RRP12
	Nucleotide metabolic process	RRM1 PKM NTSC2 MCCS2 PGM1 PAPS2 HSD17B4 NNMT
	RNA processing	DDX5 GLTSCR2 DDX21 HNRNPH3 GEMINS U2AF2 PRKDC GEMIN4 RRS1 HNRNPH1 GTPBP4 PTBP2 PUF60 RRP12
	RNA metabolic process	TRIP13 DDX5 GLTSCR2 NOTCH2 DDX21 POLR1A HNRNPH3 GEMINS U2AF2 PRKDC GEMIN4 RRS1 HNRNPH1 TTC37 GTPBP4 PTBP2 TCOF1 LARS TAR5 PUF60 RRP12 GTF2 DDX24 CSDC1
Formation of protein complex	Ribonucleoprotein complex assembly	GLTSCR2 GEMINS PRKDC GEMIN4 RRS1 PTBP2 PUF60
	Ribonucleoprotein complex biogenesis	GLTSCR2 DDX21 GEMINS PRKDC GEMIN4 RRS1 GTPBP4 PTBP2 PUF60 RRP12
	Protein-containing complex assembly	GLTSCR2 GEMINS RRM1 MAT2A PRKDC BIN1 GEMIN4 RRS1 DNAJB12 FN1 PTBP2 ATL2 DIAPH1 DIAPH3 PUF60 EHD1
Regulation of translation	rRNA processing / Ribosome biogenesis	GLTSCR2 DDX21 PRKDC GEMIN4 RRS1 GTPBP4 RRP12
Others	Pos. reg. of endocytic recycling	EHD2 EHD1
	Maintenance of dna methylation	DNMT1 UHRF1
	Response to nutrient	OXCT1 NNT AGL MAP1B PKM AAC5

SKOV-3

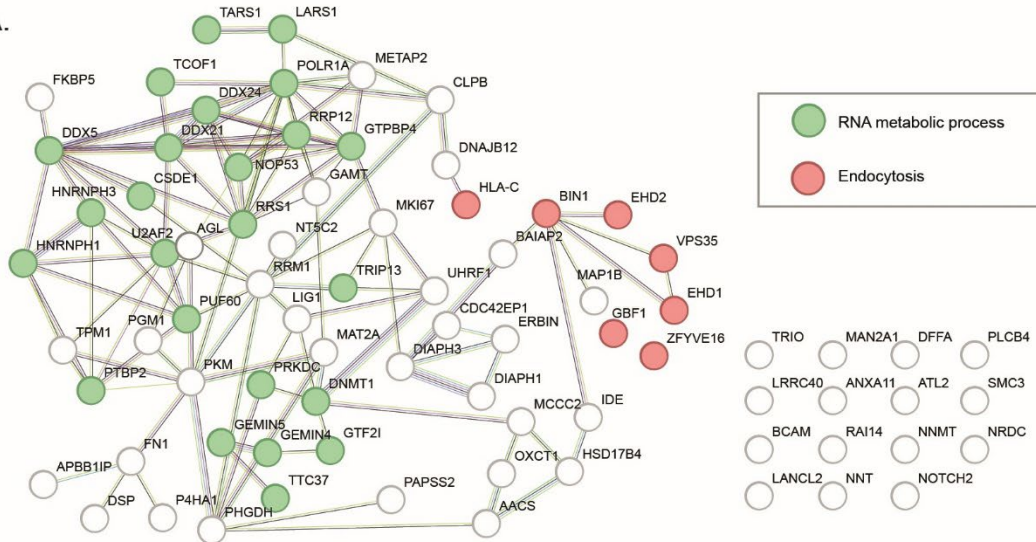
Process	Go term	Associated genes
Regulation of translation	rRNA processing	FTSJ3 BRX1 DDX54 DDX56 EIF4A3 RRP1B PRKDC
	Reg. of translation	NCL PUM1 THBS1 EIF4A3 ERBB2 APP LARP1 RBM4 PRKDC
	Ribosome biogenesis	FTSJ3 BRX1 DDX54 DDX56 EIF4A3 RRP1B PRKDC
Regulation of RNA	ncRNA processing	FTSJ3 BRX1 DDX54 DDX56 SSB EIF4A3 RRP1B PRKDC
	RNA splicing	NCL SMU1 EIF4A3 NONO RRP1B RBM4 HNRNPA0 AHNAK2
	Posttranscriptional reg. of gene expression	AAAS NCL PUM1 THBS1 EIF4A3 ERBB2 APP LARP1 RBM4 HNRNPA0 PRKDC
	RNA processing	FTSJ3 BRX1 NCL SMU1 DDX54 PUM1 DDX56 SSB EIF4A3 APP NONO RRP1B RBM4 HNRNPA0 AHNAK2 PRKDC
	mRNA metabolic process	NCL SMU1 PUM1 SSB EIF4A3 APP NONO LARP1 RRP1B RBM4 HNRNPA0
	Neg. Reg. of gene expression	AAAS NCL PUM1 THBS1 ADAM10 SSB EIF4A3 APP LARP1 AXL RBM4 HNRNPA0 PRKDC
Others	Response to epidermal growth factor	NCL ERBB2 MCM7 BAIAP2
	Extracellular matrix organization	ITGA6 COL12A1 ITGB4 THBS1 ADAM10 APP SERPINH1 AGRN
	Reg. of cellular amide metabolic process	NCL PUM1 THBS1 EIF4A3 ERBB2 APP LARP1 RBM4 PRKDC
	Ribonucleoprotein complex biogenesis	HSP90AA1 FTSJ3 BRX1 DDX54 DDX56 EIF4A3 RRP1B PRKDC
	Cell junction organization	ITGA6 ITGB4 THBS1 ADAM10 ERBB2 APP PTPRF BAIAP2 AGRN L1CAM
	Cellular component morphogenesis	HSP90AA1 ITGB4 ADAM10 ERBB2 APP CDC2C KRT8 KRT19 BAIAP2 L1CAM
	Neurogenesis	HSP90AA1 ITGA6 ITGB4 DDX56 ADAM10 ERBB2 APP PTPRF CDC2C AXL BAIAP2 AGRN L1CAM TGM2

Supplementary Figure 3. Biological processes associated with the proteins altered when HOOK1 is downregulated. Biological processes, GO terms and associated genes of (A) upregulated and (B) downregulated proteins in OVCAR-8 and SKOV-3 cell lines with downregulated HOOK1.

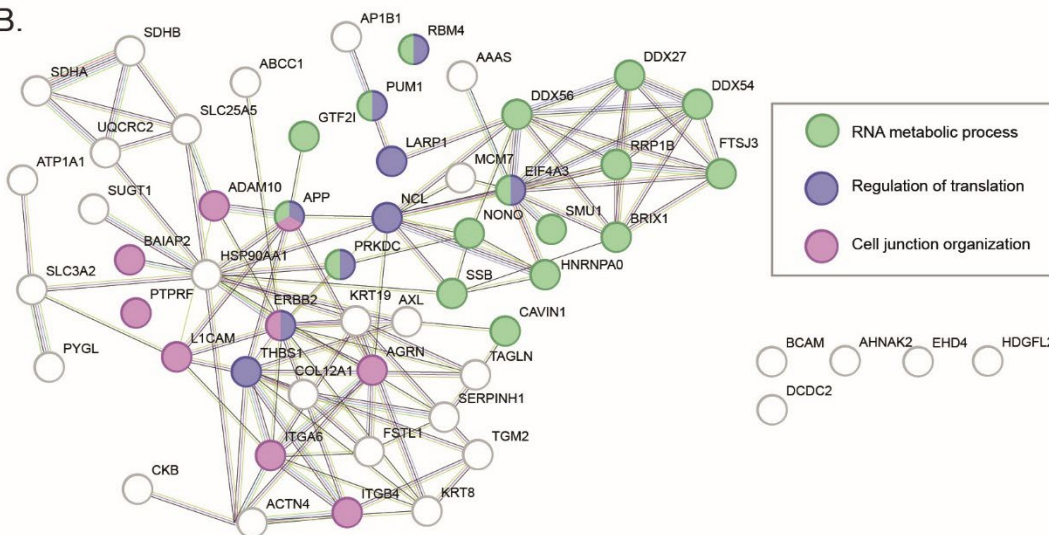


Supplementary Figure 4. Analysis of upregulated proteins upon HOOK1 downregulation using STRING resource. Network of upregulated proteins in **(A)** OVCAR-8 and **(B)** SKOV-3 cell line. Network nodes represent proteins and edges represent protein-protein associations. Some genes are colored to show that they are associated to a specific GO term. **(C)** Network of the top 5 most significantly upregulated proteins in OVCAR-8 and SKOV-3 cell lines and brief description of their function within the cells.

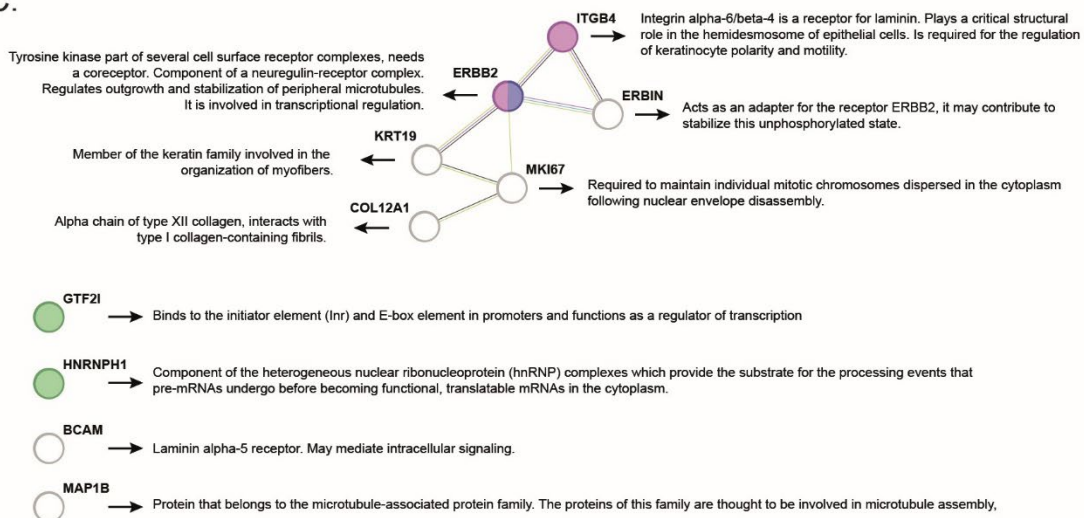
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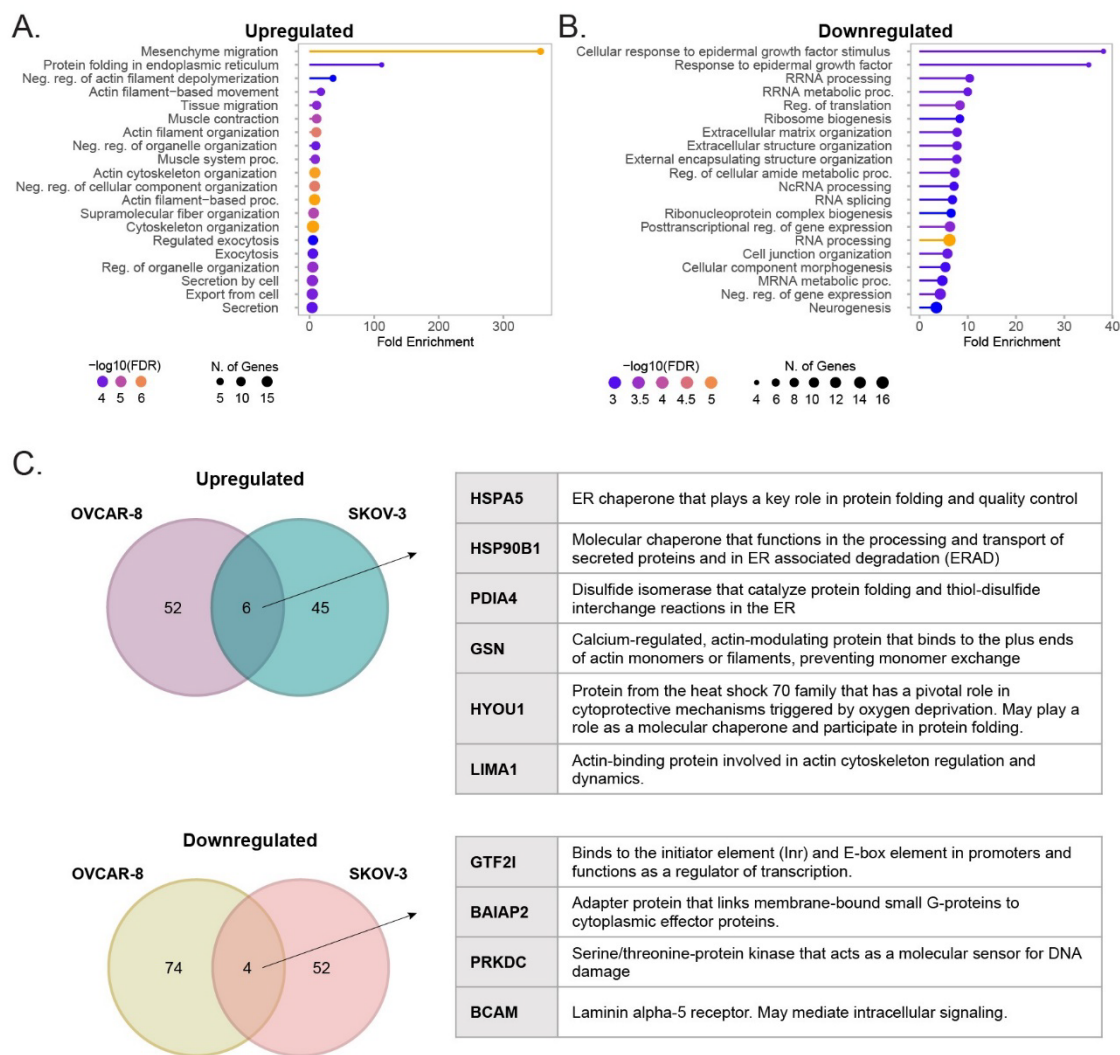
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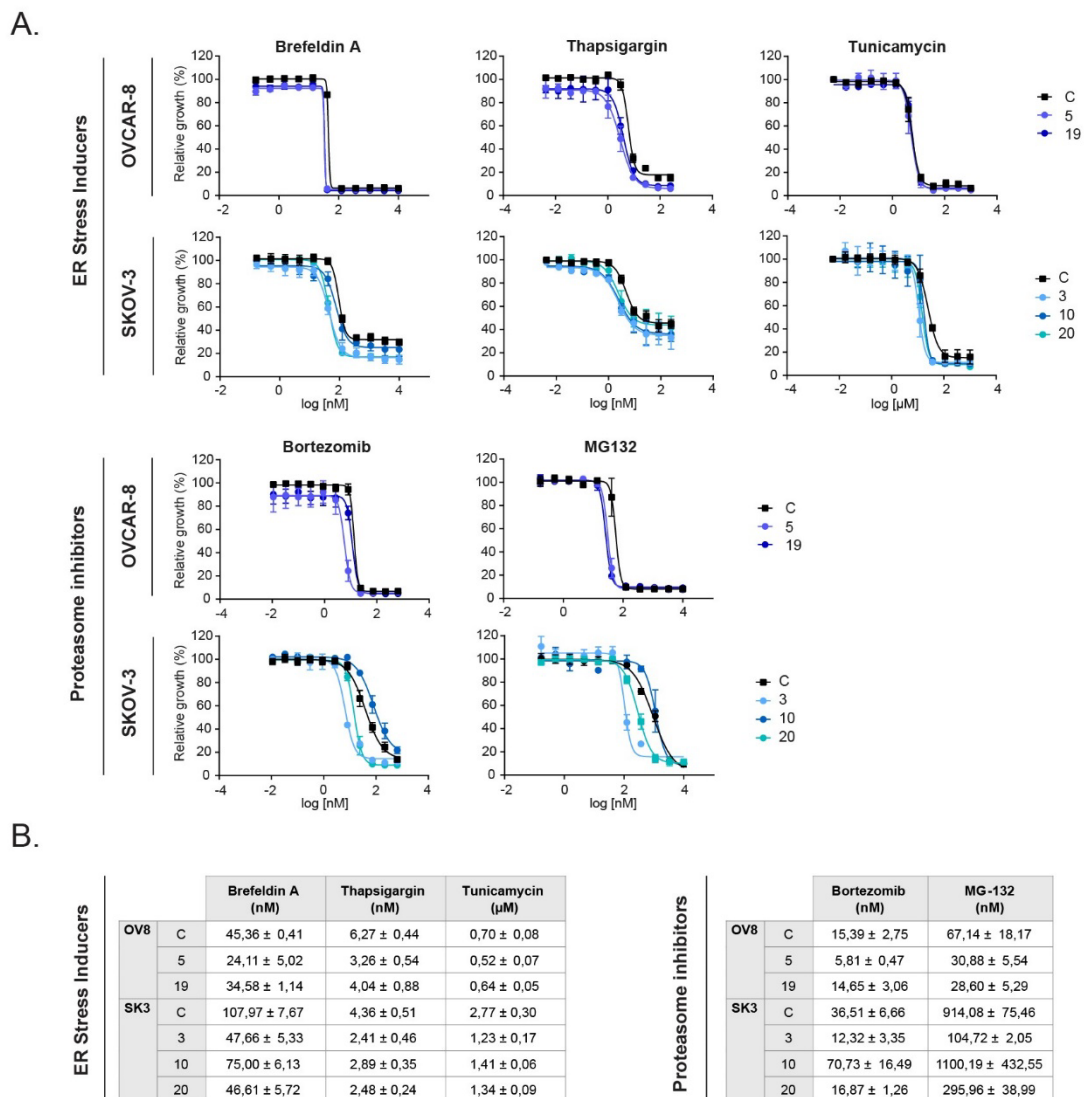
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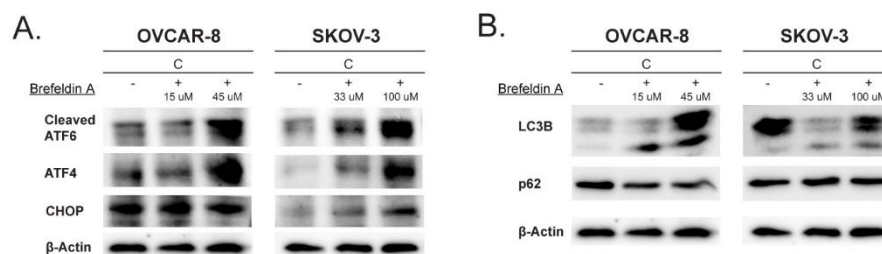
Supplementary Figure 5. Analysis of downregulated proteins upon HOOK1 downregulation using STRING resource. Network of downregulated proteins in **(A)** OVCAR-8 and **(B)** SKOV-3 cell line. Network nodes represent proteins and edges represent protein-protein associations. Some genes are colored to show that they are associated to a specific GO term. **(C)** Network of the top 5 most significantly downregulated proteins in OVCAR-8 and SKOV-3 cell lines and brief description of their function within the cells.



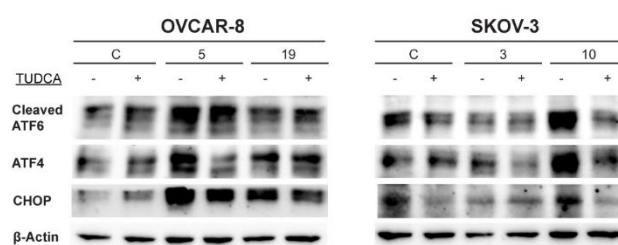
Supplementary Figure 6. Analysis of Gene Ontology and overlapping genes upon Hook1 downregulation. Gene Ontology terms of **(A)** upregulated ($FC > 1$) and **(B)** downregulated ($FC < -1$) proteins upon Hook1 downregulation in the SKOV-3 cell line. **(C)** Venn diagram of upregulated and **(D)** downregulated proteins in OVCA8 and SKOV-3 cell lines with downregulated HOOK1. A brief description of the overlapping genes is shown.



Supplementary Figure 7. IC50 curves and mean values upon Hook1 downregulation. (A) IC50 curves of compounds that induce ER stress or inhibit proteasome function in Hook1-downregulated cells. The mean of 3 independent experiments ± SEM is represented. **(B)** Table with mean values of IC50 ± SEM of cells with downregulated HOOK1 treated with ER stress inducers or proteasome inhibitors.

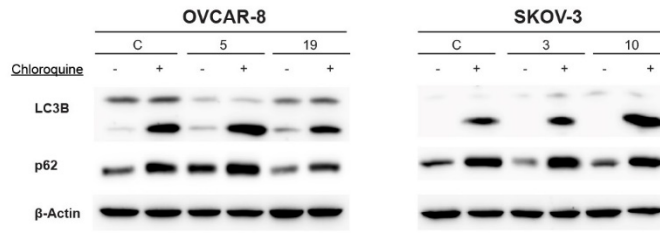


Supplementary Figure 8. Validation of brefeldin A treatment. (A) Protein levels of UPR-associated proteins in Hook1-downregulated cells treated with brefeldin A. (B) Protein levels of autophagy-associated proteins in Hook1-downregulated cells treated with brefeldin A.

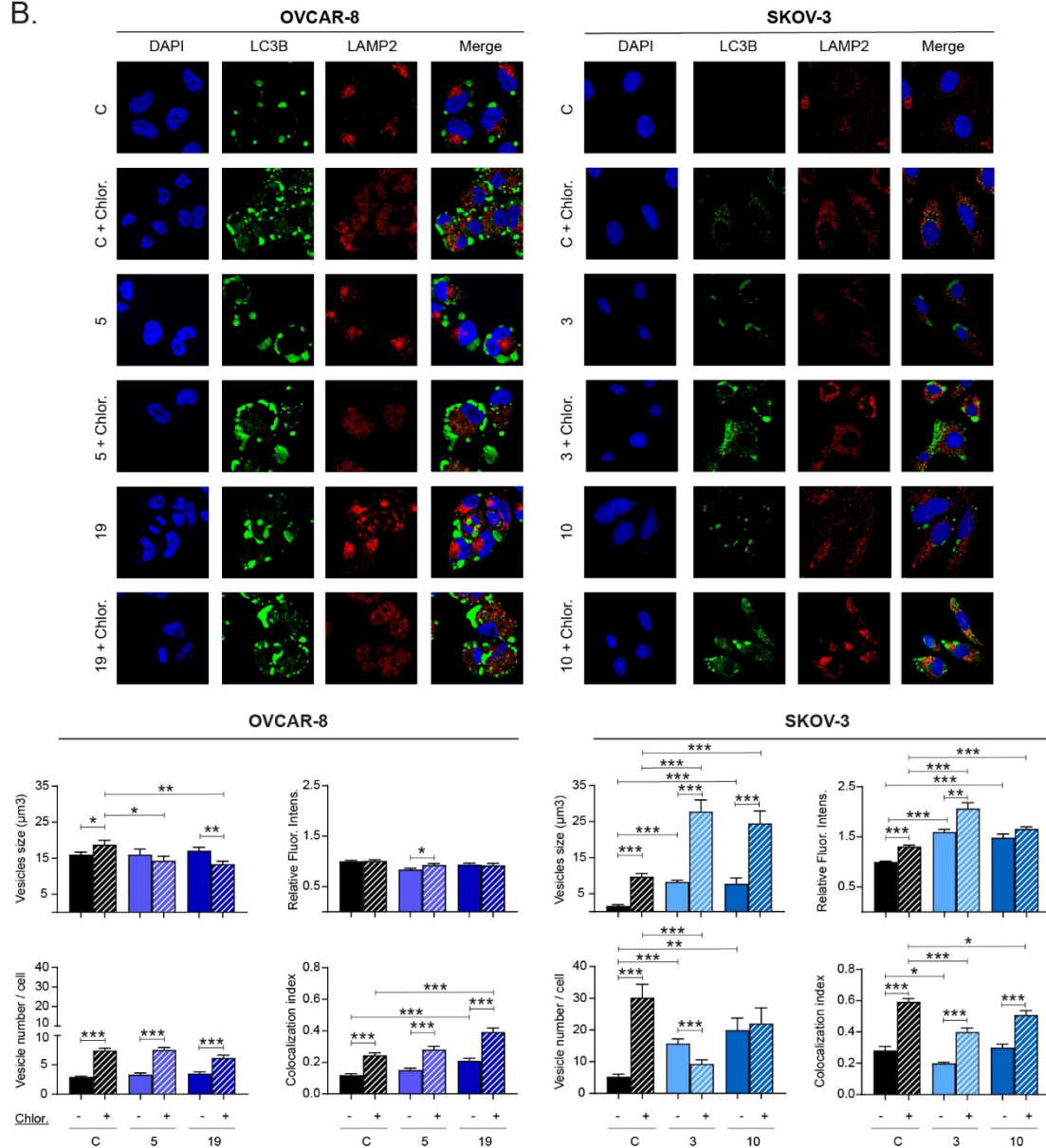


Supplementary Figure 9. Validation of TUDCA treatment. Protein levels of UPR-associated proteins in Hook1-downregulated cells treated with TUDCA.

A.

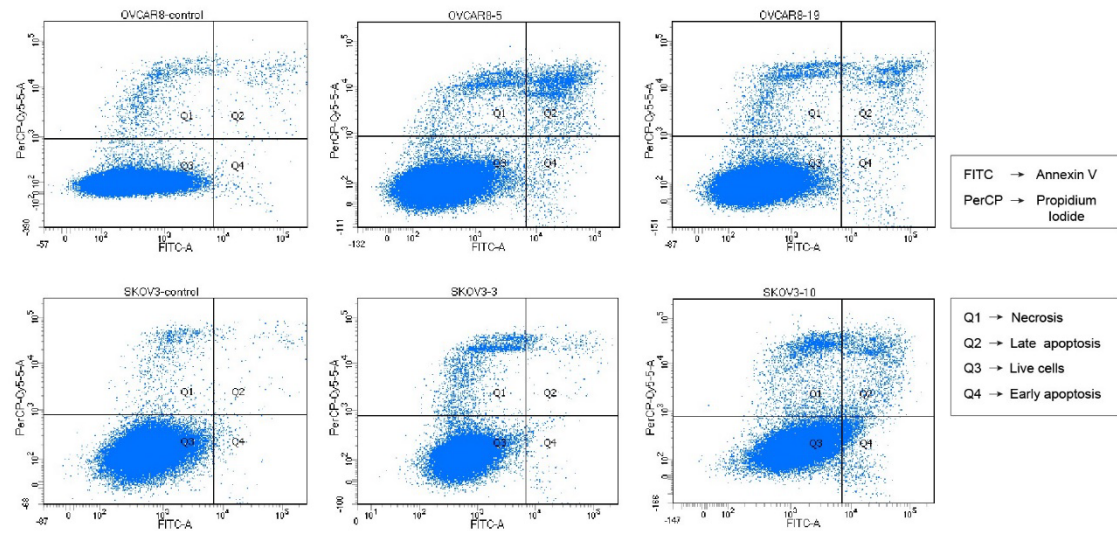


B.



Supplementary Figure 10. Validation of chloroquine treatment. (A) Protein levels of autophagy-associated proteins in Hook1-downregulated cells treated with chloroquine. (B) Immunofluorescence staining of LC3B (green) and LAMP2 (red) proteins in Hook1-downregulated cells treated with chloroquine. DAPI (blue) was

used for nuclear staining, and the merge of the 3 markers is shown. The autophagosome size, number and relative fluorescence intensity, as well as the colocalization index of autophagosomes-lysosomes, were measured using ImageJ software. The statistical analysis was performed using Student's t test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).



Supplementary Figure 11. Representative images of the cell death assay. Flow cytometry charts of ovarian cancer cells with downregulated HOOK1 stained with Annexin V and propidium iodide.

Treatment	Origin	Concentration OVCAR-8 line	Concentration SKOV-3 line
Brefeldin A	MedChemExpress	45 nM	100 nM
Chloroquine	MedChemExpress	25 μ M	50 μ M
TUDCA (tauroursodeoxycholic acid)	MedChemExpress	100 μ M	100 μ M

Supplementary Table 1. Concentration of the treatments employed in the ovarian cancer cell lines.

Figure 1D

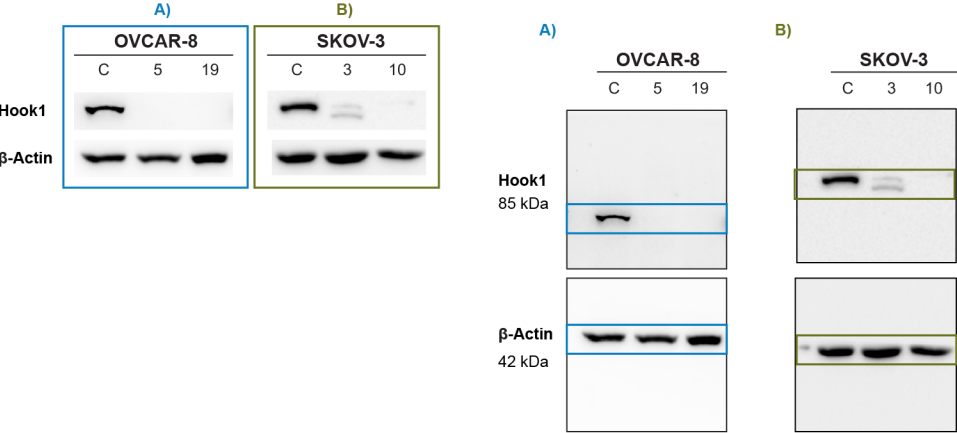


Figure 3D

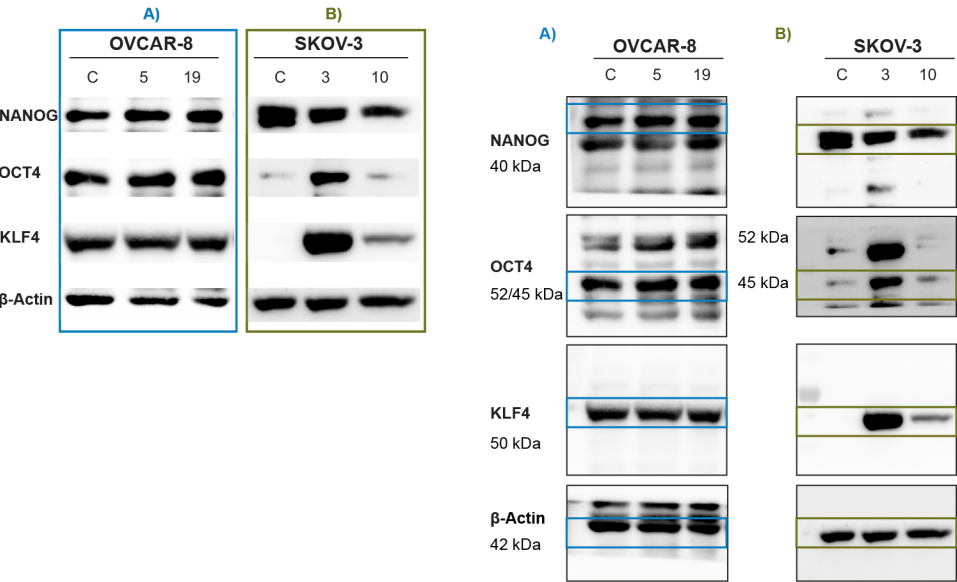


Figure 4E

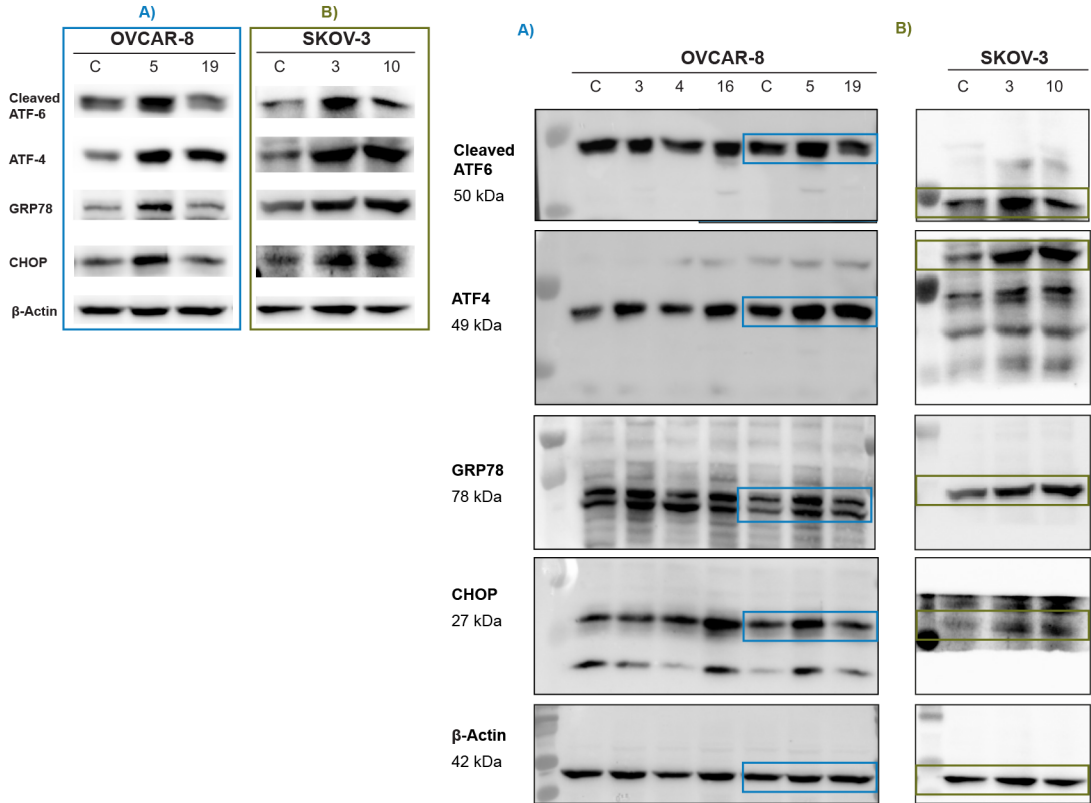


Figure 5A

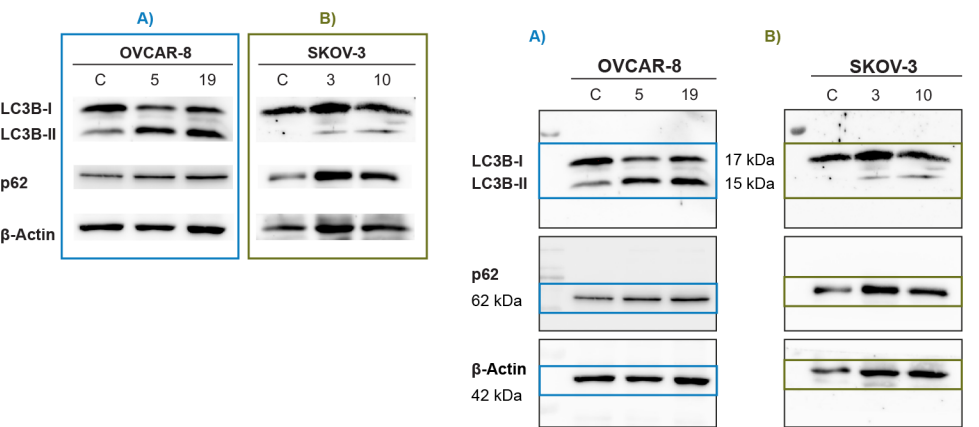


Figure 2C

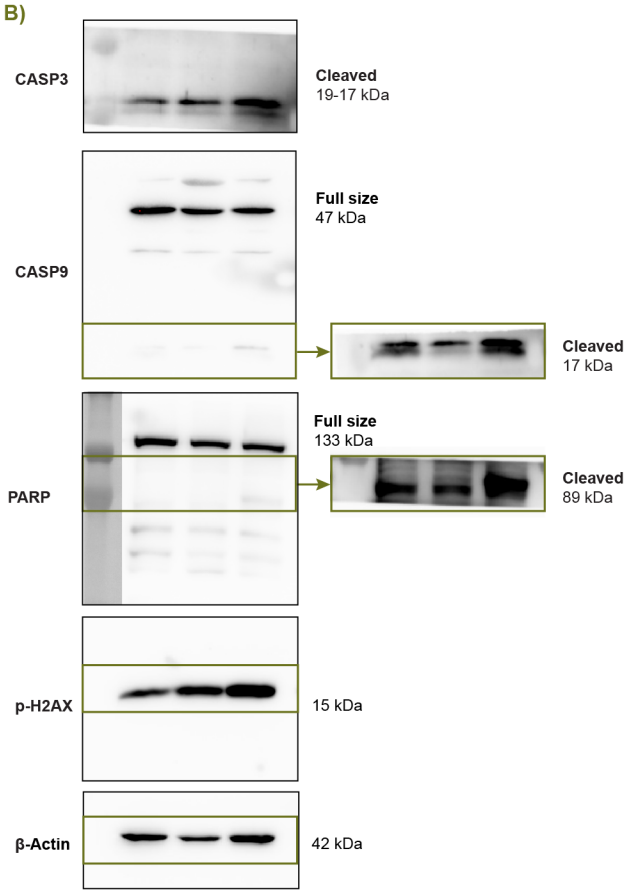
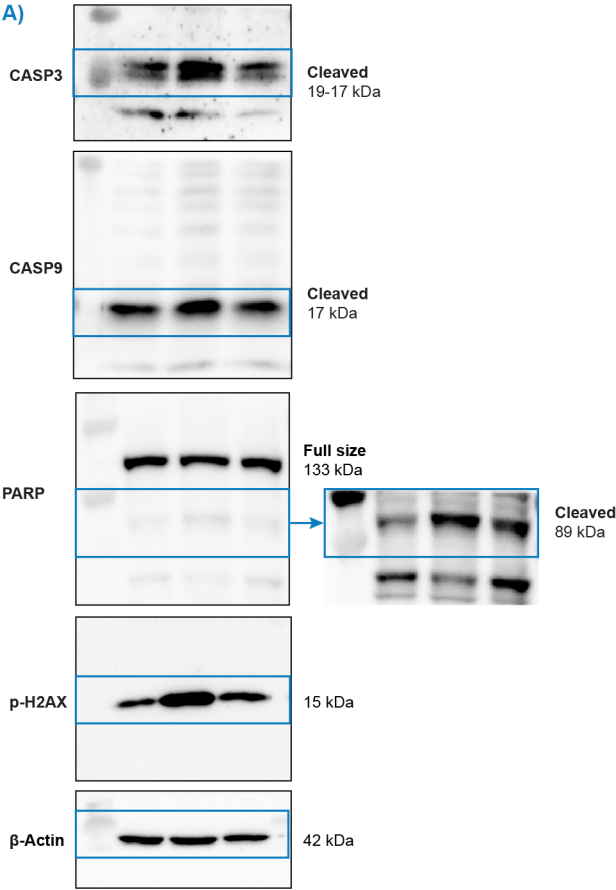
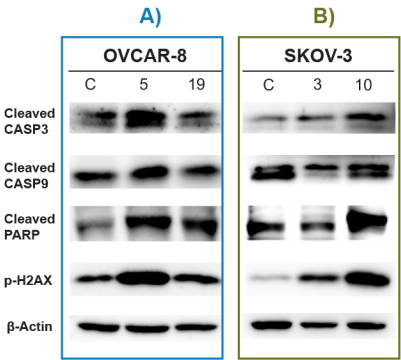


Figure 6A

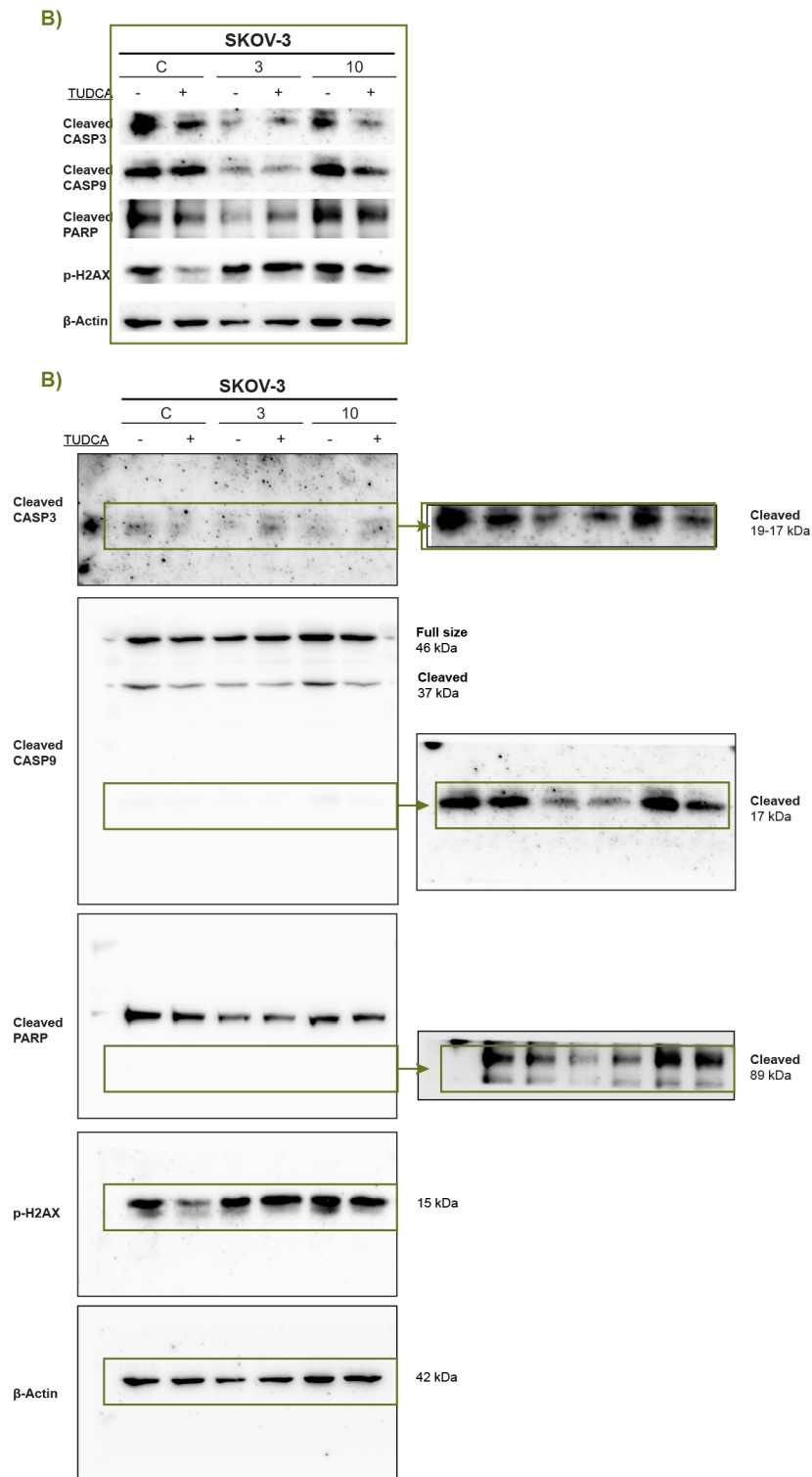
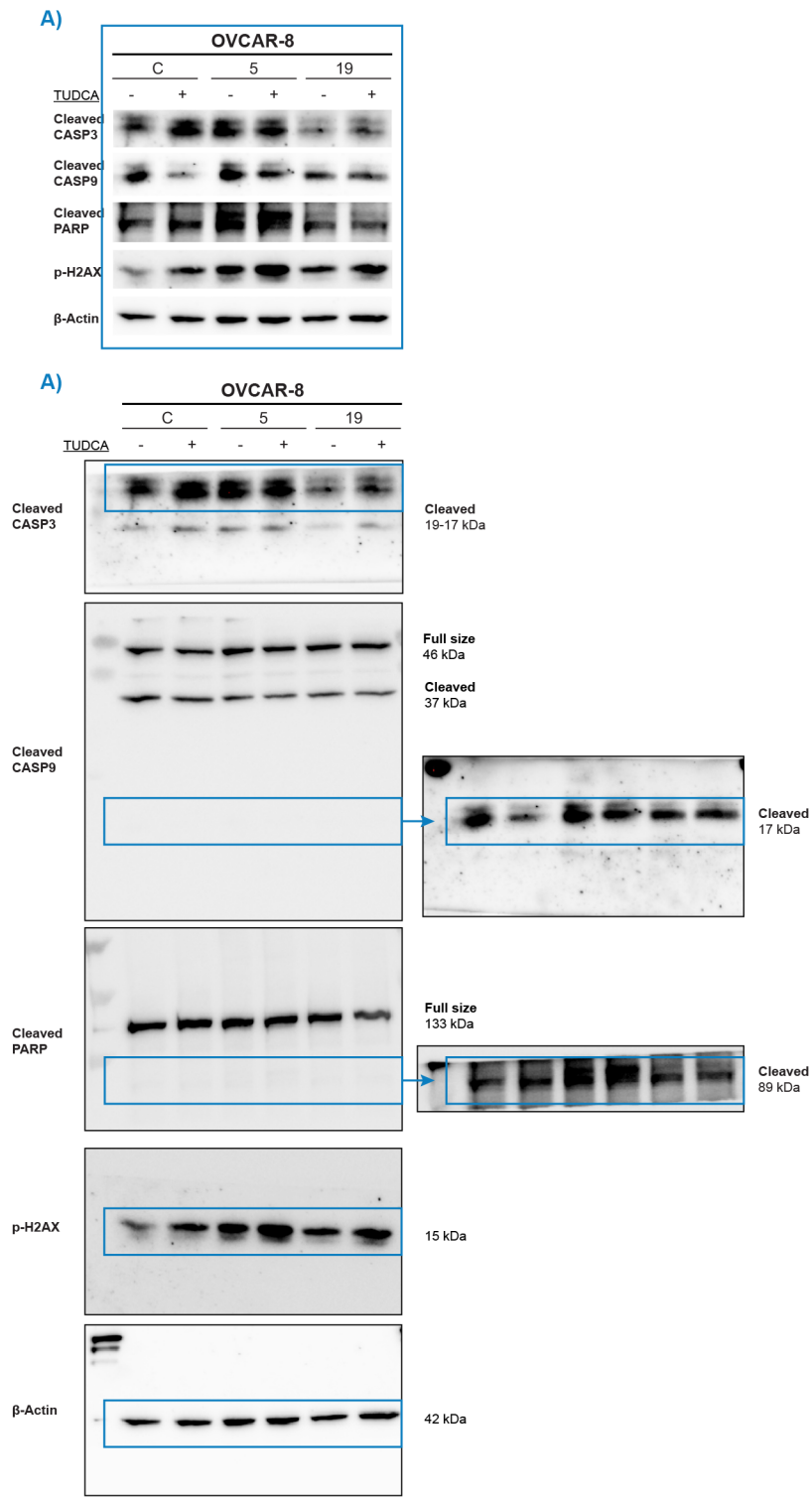


Figure 6B

