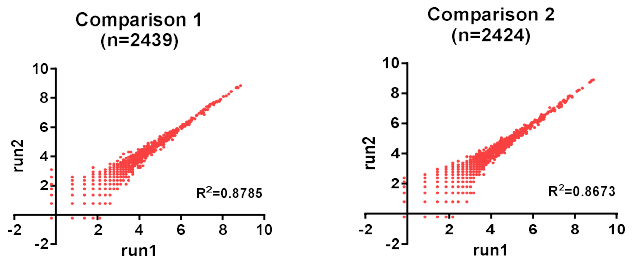
Supplemental figures

FIG S1

A.



B.

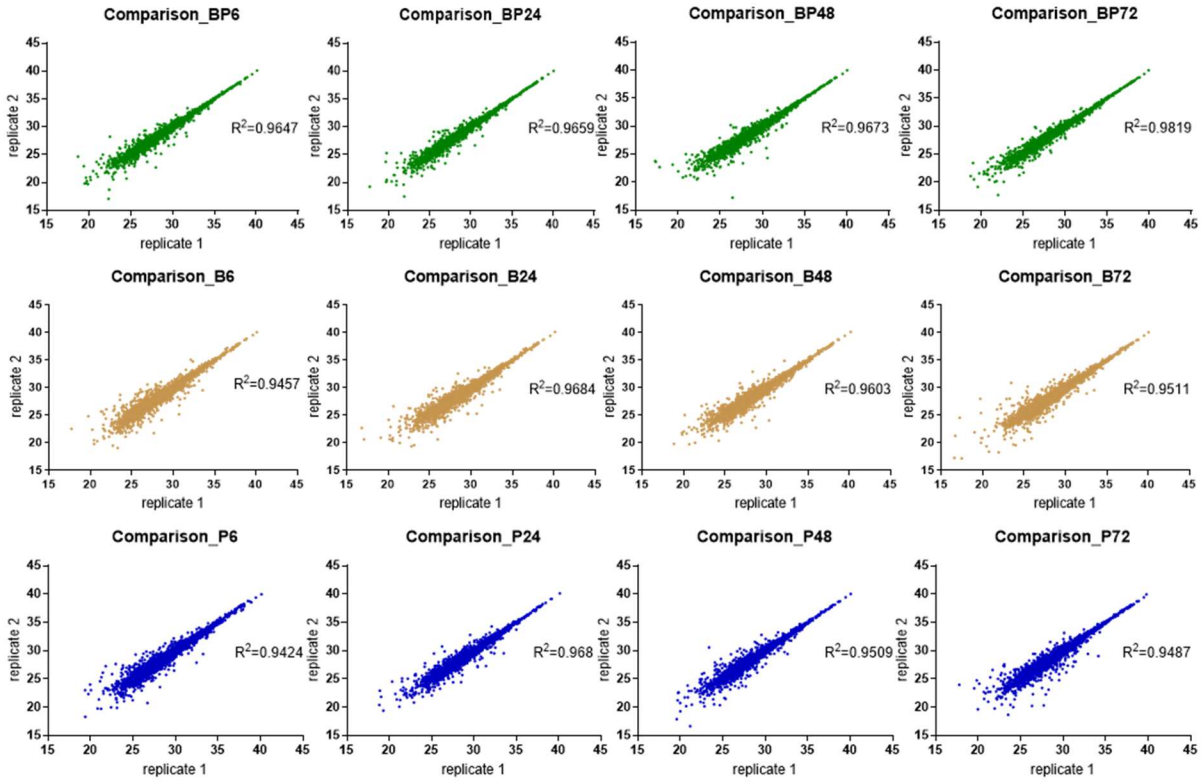
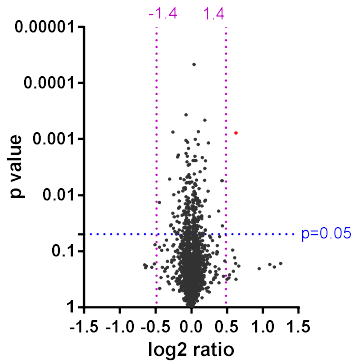


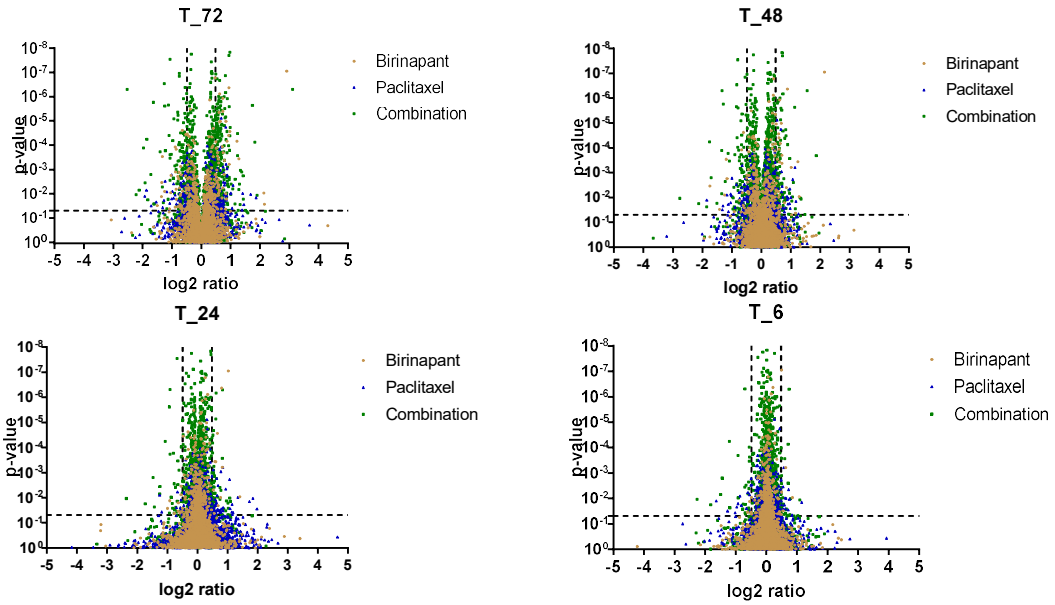
FIG S1. Further investigation of quantitative consistency. (A) Reproducibility of SpC-based quantification in technical replicates. In addition to ion current-based quantification, proteins from the experimental null dataset were also quantified based on spectral counts for comparison. The log2 intensities of quantified proteins from two replicate runs of the same sample were analyzed by linear regression to evaluate the reproducibility of the quantitative data. Much inferior reproducibility of SpC-based quantification were observed compared to ion current-based quantification. **(B) Correlations of the protein quantitative values by IonStar between two randomly-selected biological replicates in each biological group**.

FIG S2

A.



B.



Significantly altered proteins in each group												
	Birinapant				Paclitaxel				Combination			
	T6	T24	T48	T72	T6	T24	T48	T72	T6	T24	T48	T72
Upregulation	6	13	36	53	15	46	40	78	28	56	112	251
Downregulation	5	11	19	44	13	9	42	63	24	40	85	142
Total altered proteins	11	24	55	97	28	55	82	141	52	96	197	393

FIG S2. Volcano plots of protein ratios vs. *p-values* in the Experimental Null dataset and all experimental groups. (A) The dotted lines indicate the optimized cut-off thresholds: 1.4-fold (log2 ratio range from -0.4854 to 0.4854) and p=0.05. The red dots indicate the false positives under the given thresholds; only 1 false positive was discovered and the measured FADR was <1%. (B) The volcano plots (expression ratios vs. p-values of all quantified proteins) with 3 treatments at 4 time points. The dotted line represent cut-off thresholds determined by the EN method. The numbers of significantly altered proteins in each experimental group were shown in the bottom table.

FIG S3

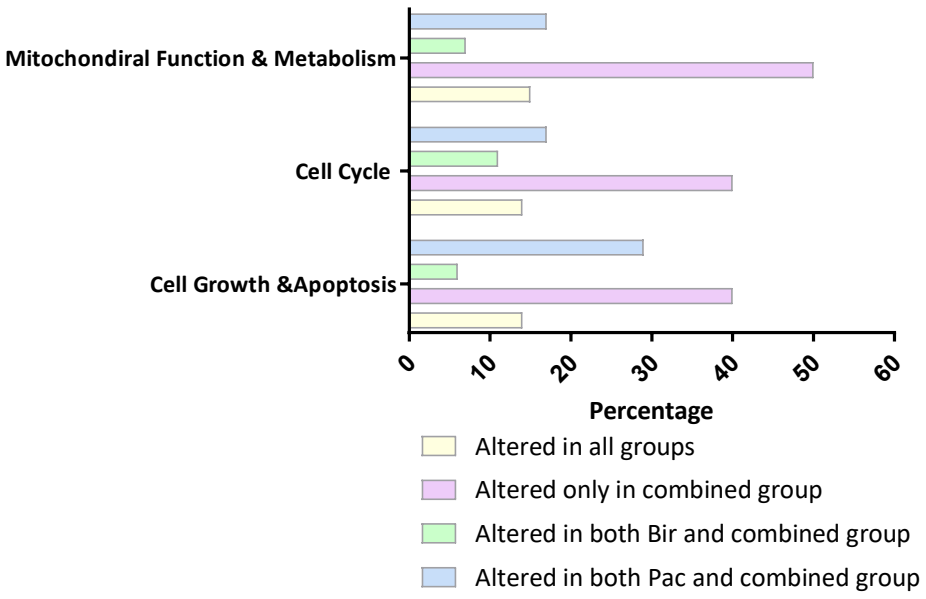


FIG S3. Distribution of altered proteins in three biological processes. For mitochondrial function and metabolism associated altered proteins, >50% were exclusively altered by combined birinapant/paclitaxel. For cell growth associated altered proteins, ~ 40% were discovered only in combined group. For cell cycle progression associated altered proteins, ~40% of them were altered uniquely by combination treatment.

FIG S4

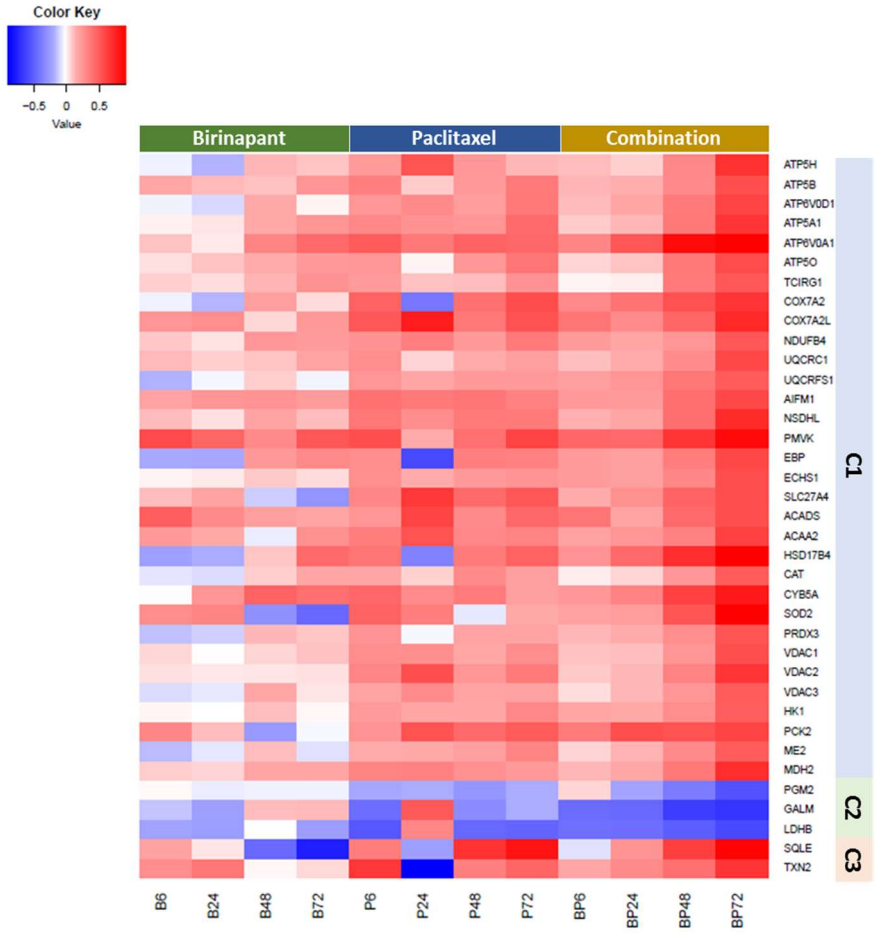


FIG S4. Quantification and clustering of altered proteins that are unique to combined birinapant/paclitaxel treatment and associated with mitochondrial function and metabolism. The quantified proteins associated with mitochondrial dysfunction are grouped in the heatmap into three clusters based on k-means clustering algorithm. The color key represents the log2 ratio of treated groups compared to control. Cluster 1 and cluster 3 represent proteins that were exclusively increased in the drug combination group compared to single agents, whereas cluster 2 contains proteins that were decreased significantly in the drug combination group compared to single drug-treated groups.

FIG S5

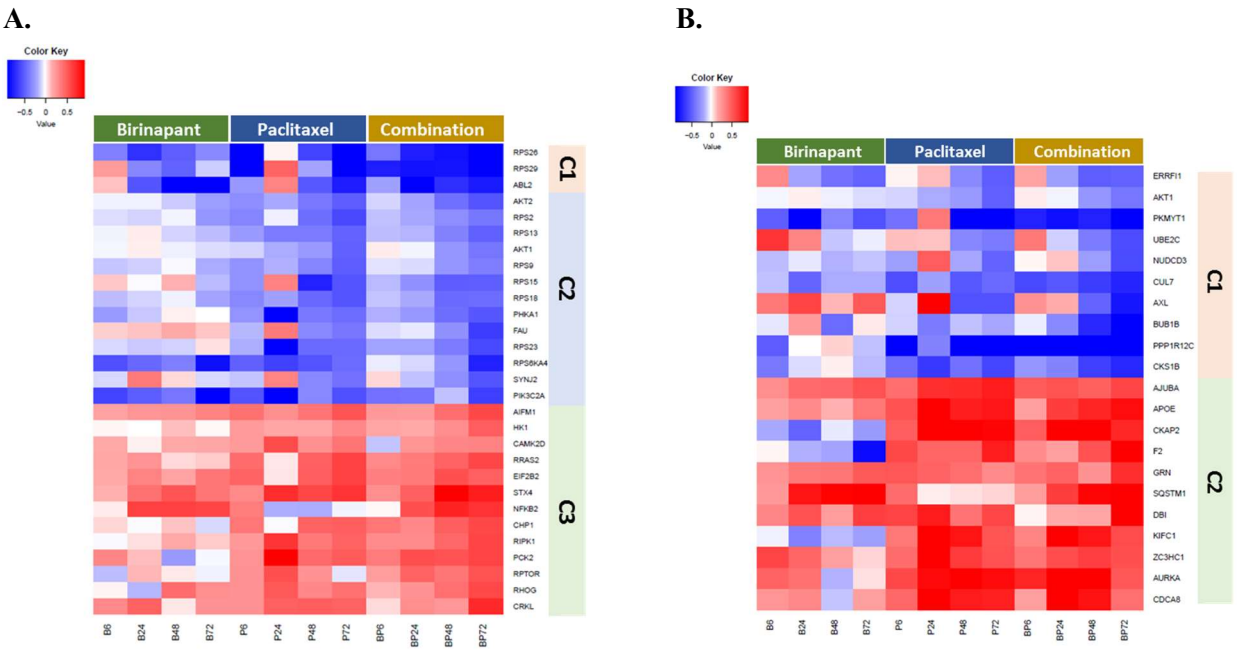


FIG S5. Quantification and clustering of altered proteins that are unique to the combination drug treatment and involved in cell growth and cell cycle progression. (A) Heatmap of altered proteins involved in cell growth and apoptosis and exclusively altered in the drug combination group. Color denotes extents of change relative to untreated controls. These proteins were categorized into 3 clusters based on k-means clustering. Proteins in both cluster 1 and cluster 2 were downregulated significantly in the drug combination group, whereas proteins in cluster 3 were upregulated significantly in the drug combination group. (B) Ratios of altered proteins associated with cell cycle progression are grouped into two clusters. Cluster 1 includes proteins significantly downregulated by combined birinapant/paclitaxel treatment whereas cluster 2 includes proteins significantly increased by the drug combination.

FIG S6

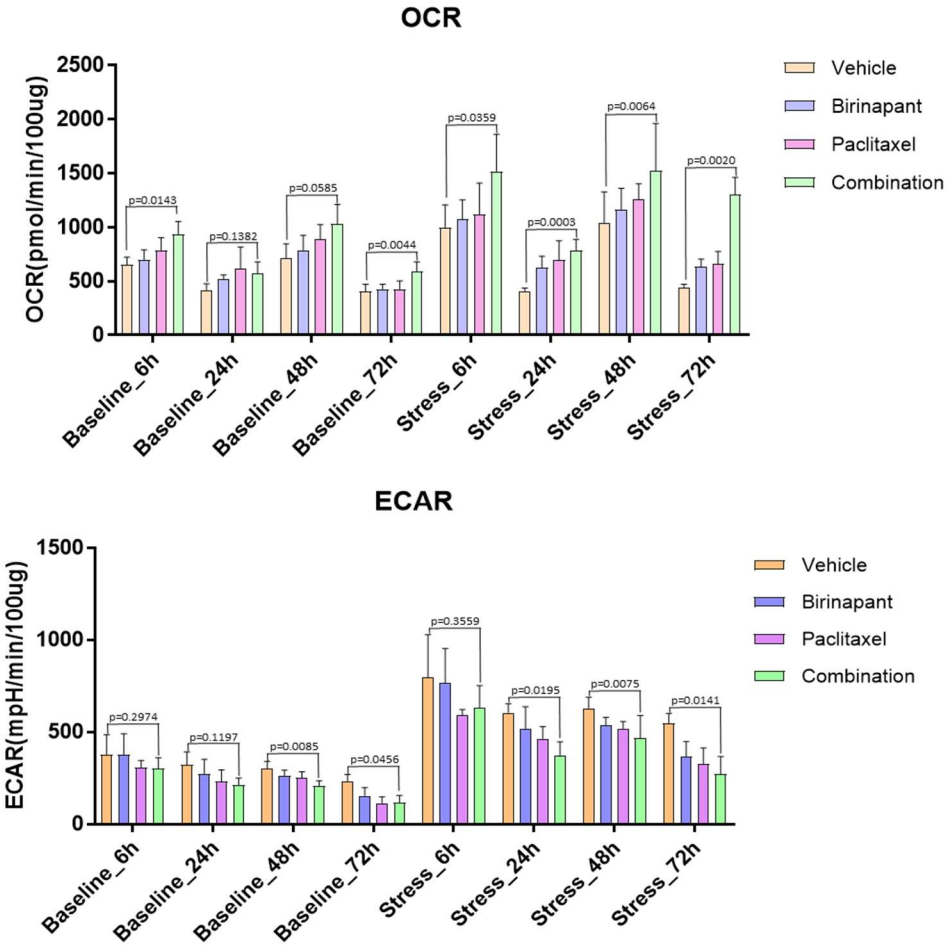


FIG S6. OCR and ECAR levels in baseline and stressed conditions before normalization. Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) of pancreatic cancer cells were measured under both baseline and stressed conditions, respectively. Stressors were oligomycin and FCCP. Under both baseline and stressed conditions, combined birinapant/paclitaxel increased OCR to a greater extent than either drug alone, while decreasing ECAR. Statistical significance was analyzed with student's t-test.

FIG S7

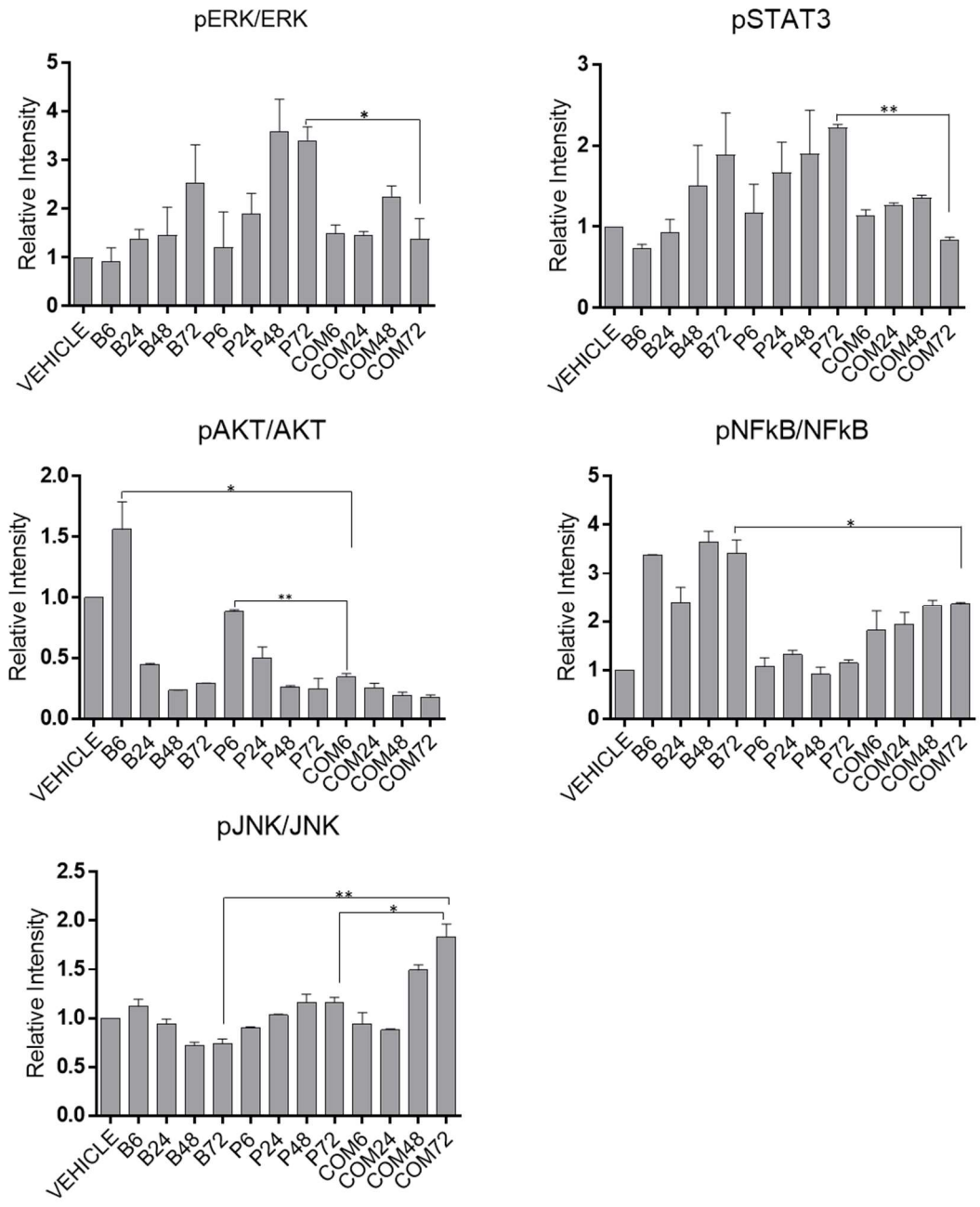
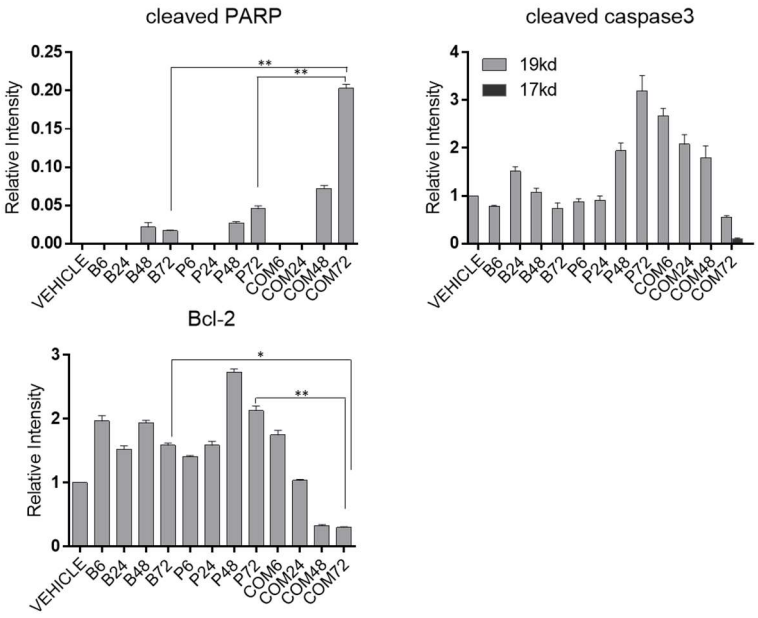


FIG S7. Quantitative analysis of altered proteins associated with cell growth-related signaling pathways. Quantification of protein phosphorylation and abundance for ERK, STAT3, AKT, NFκB, JNK for the three treatment groups (B=birinapant, P=paclitaxel, COM=birinapant/paclitaxel), and statistical significance is denoted by asterisks (* $p < 0.05$, ** $p < 0.01$).

FIG S8

A.



B.

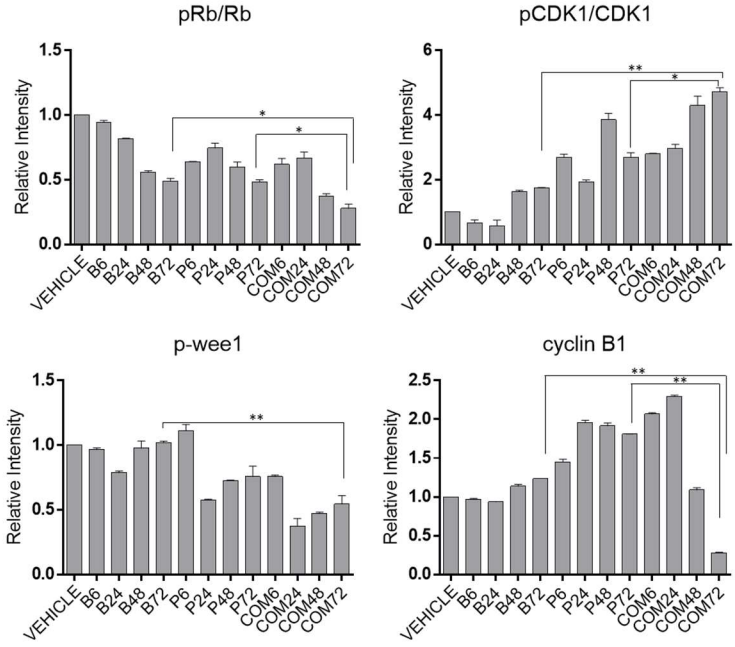


FIG S8. Quantitative analysis of altered proteins associated with apoptosis and cell cycle arrest. (A) Quantification of cleaved PARP, caspase3, and Bcl-2 was performed for the three treatment groups (B=birinapant, P=paclitaxel, COM=birinapant/paclitaxel) and statistical significance was evaluated with student's t-test. The statistical significance is denoted by asterisks (* $p < 0.05$, ** $p < 0.01$). (B) Quantitative analysis of Rb, CDK1, phosphorylated wee1 and cyclin B1, and the statistical significance is denoted by asterisks as in (A).