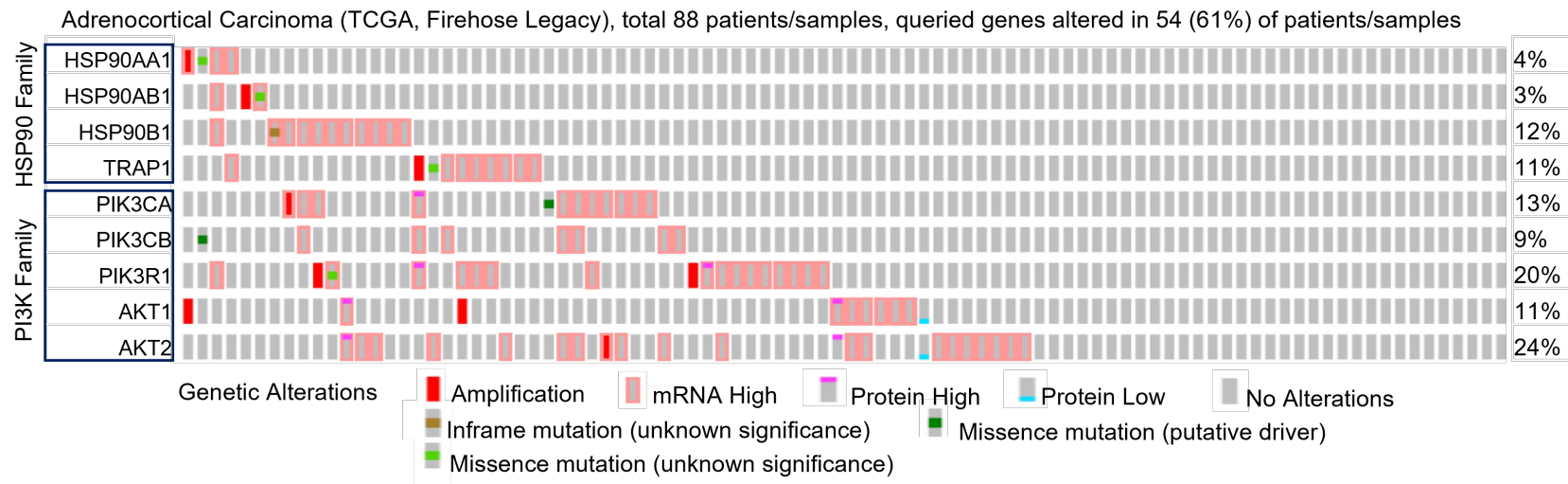
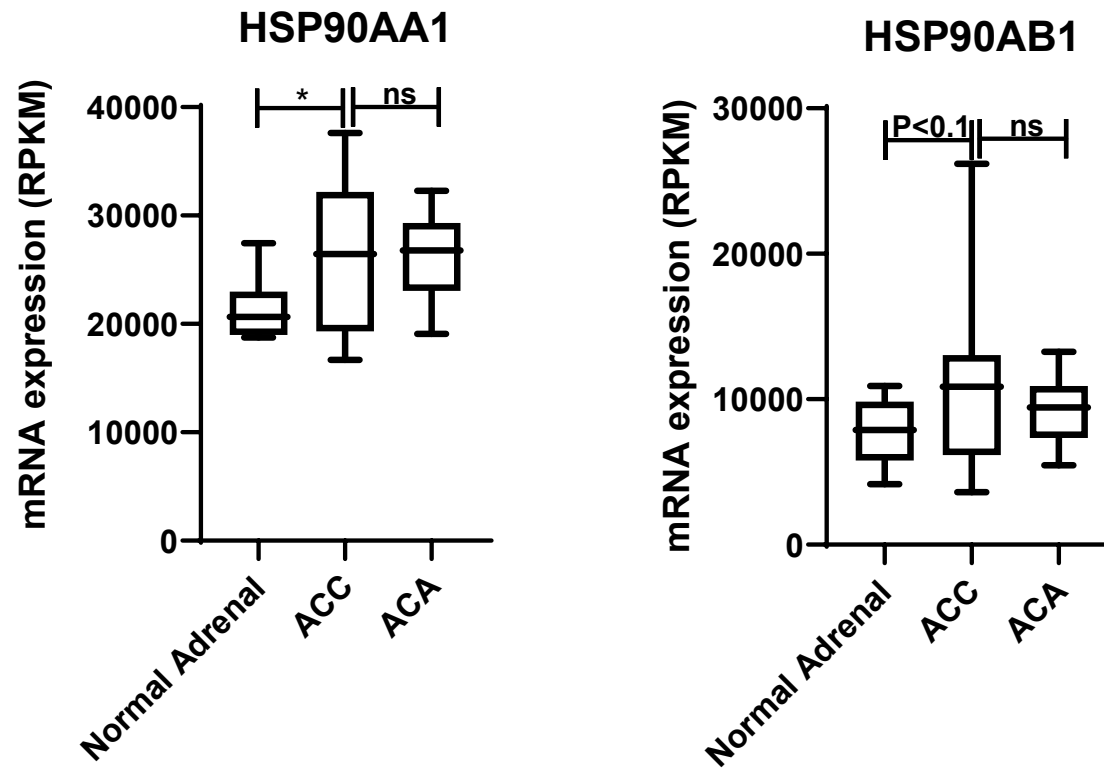


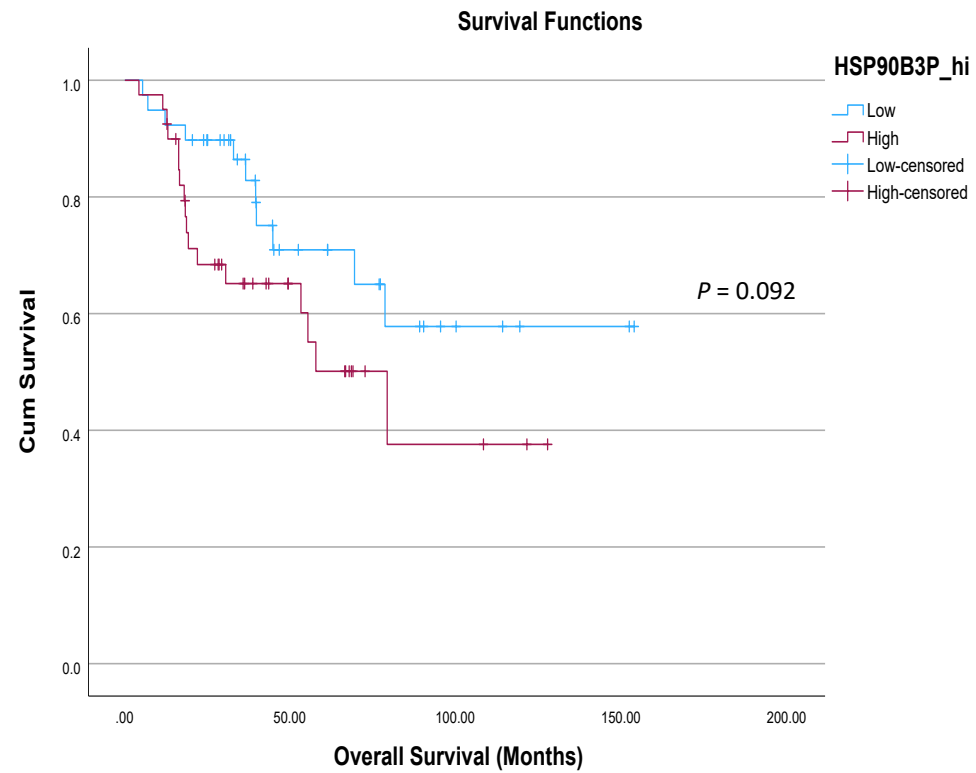


Supplementary Figure 1. Genetic alterations of HSP90 and PI3K/AKT/mTOR pathway members in ACC (TCGA, 88 patients/samples). Queried genes are altered in 54 (61%) of queried patients/samples. Each of the rectangular box in the figure represent each patient, alterations are indicated by colored boxes and grey boxes represent no changes. cBioportal Version v3.4.3 <https://www.cbioportal.org/>.

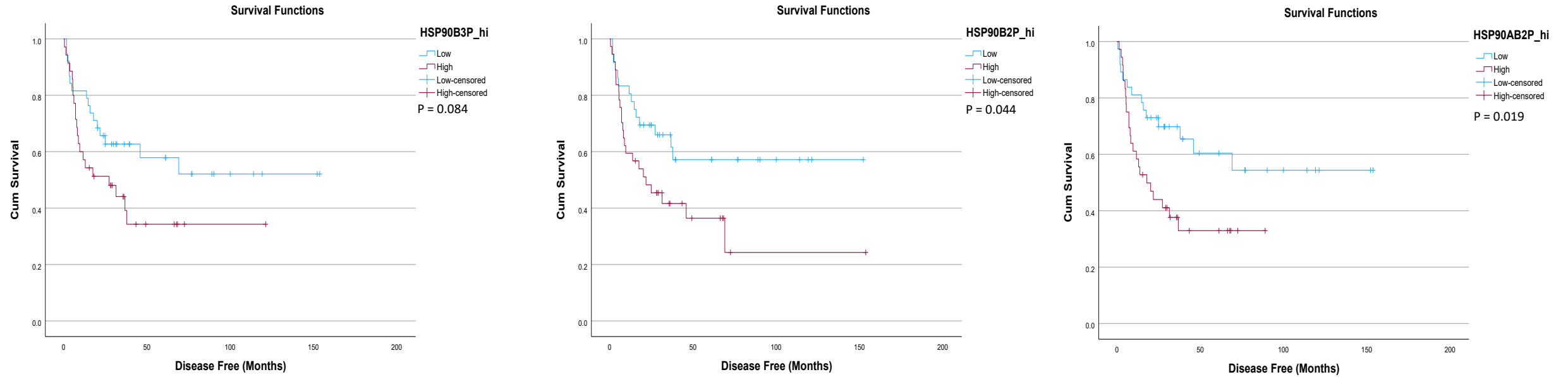
GSE12368



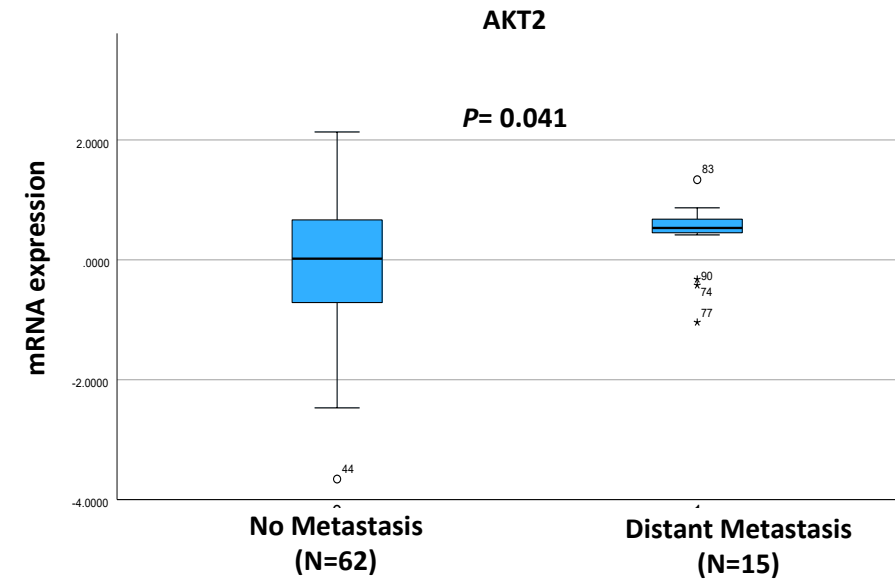
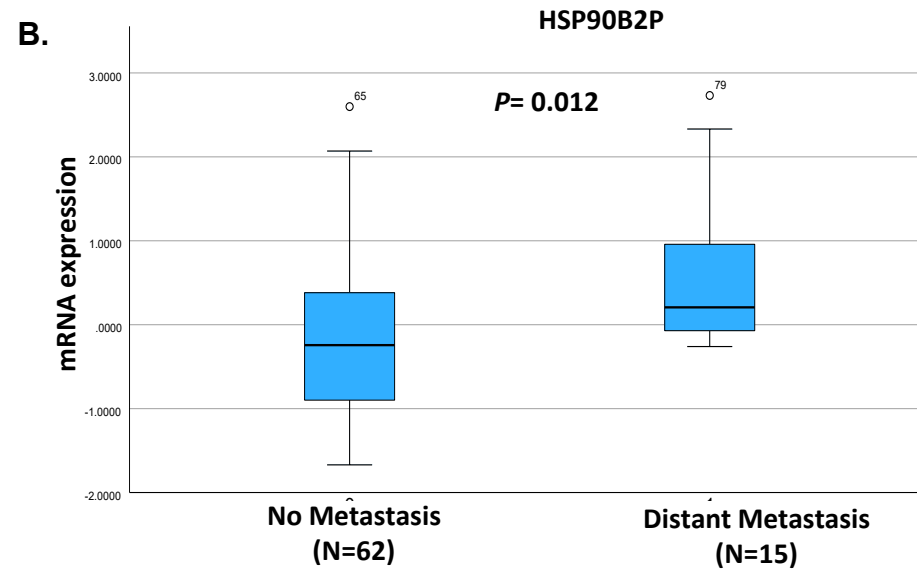
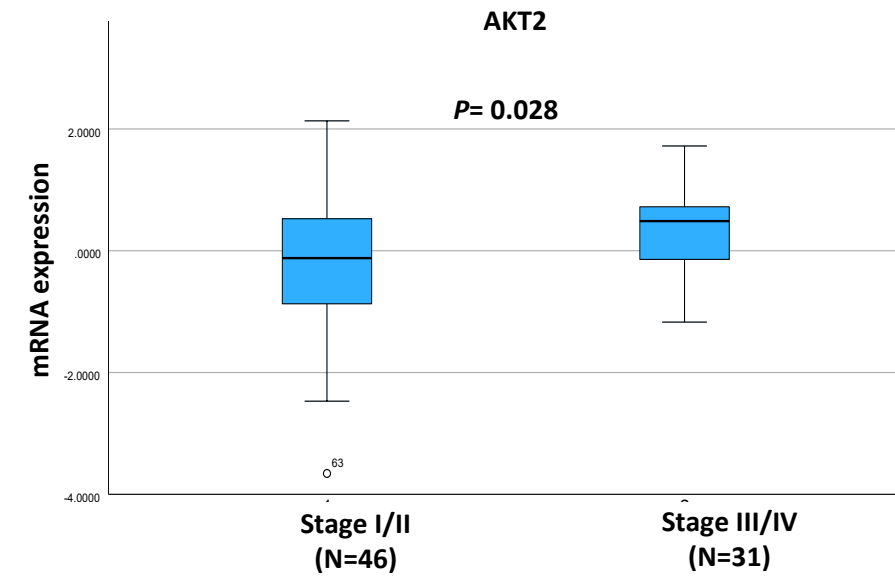
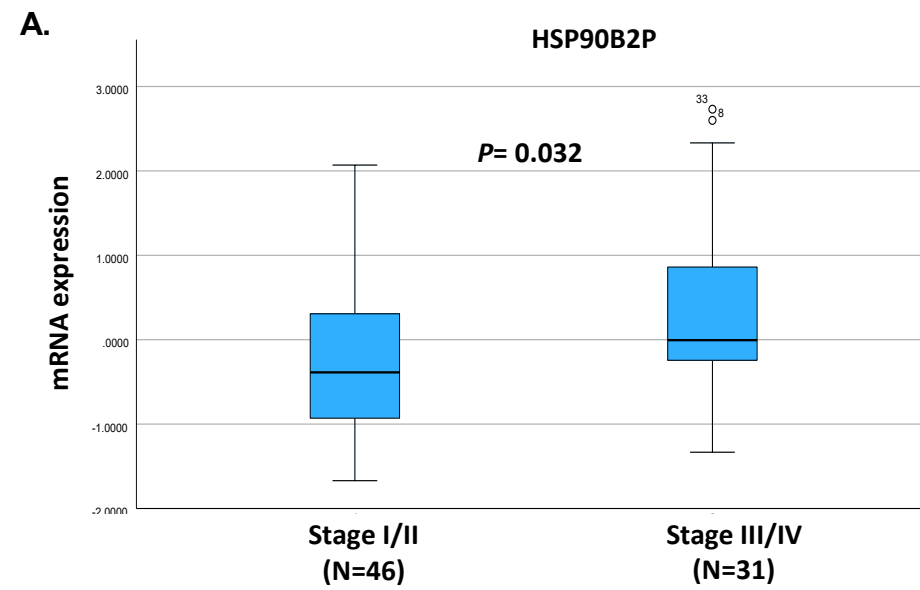
Supplementary Figure 2. Increased mRNA expression of HSP90AA1 and HSP90AB1 in adrenocortical carcinoma (n=12) and adrenocortical adenoma (n= 16) group in comparison to the normal adrenal n=6) group in the Gene Expression Omnibus (GEO) dataset GSE12368. * $P \leq 0.05$; ns, not significant.



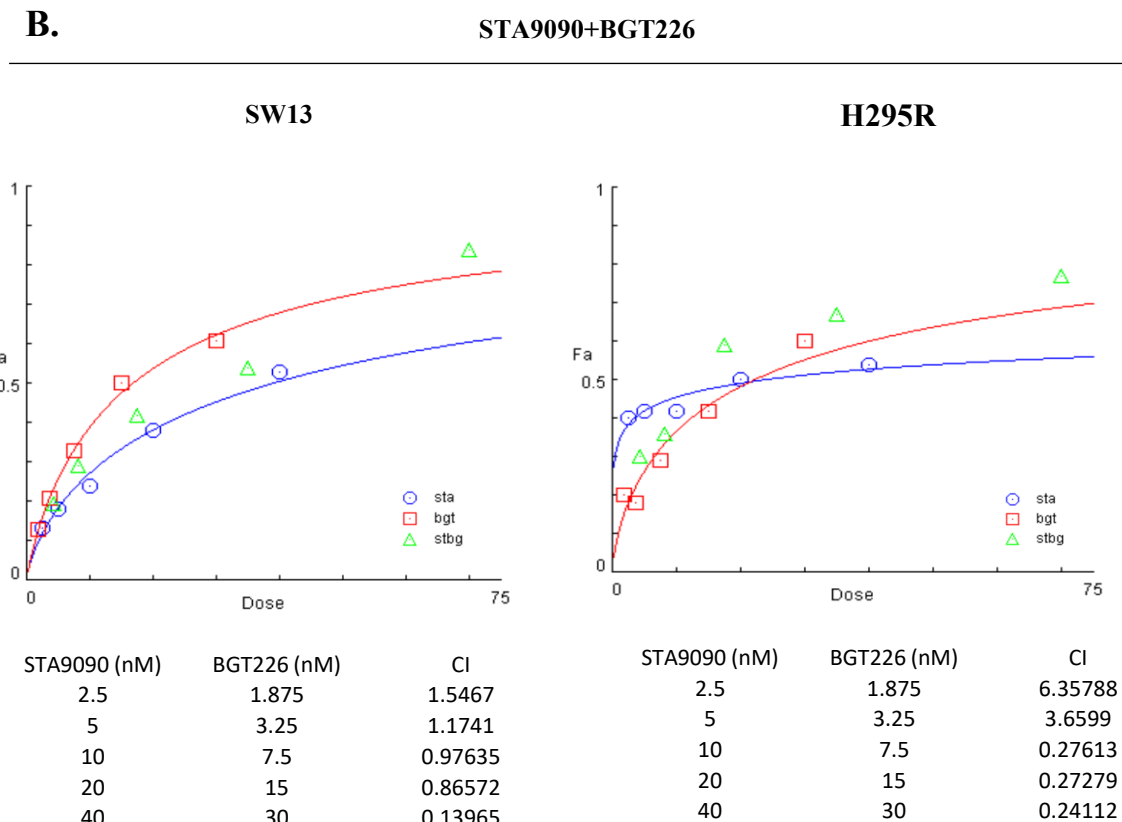
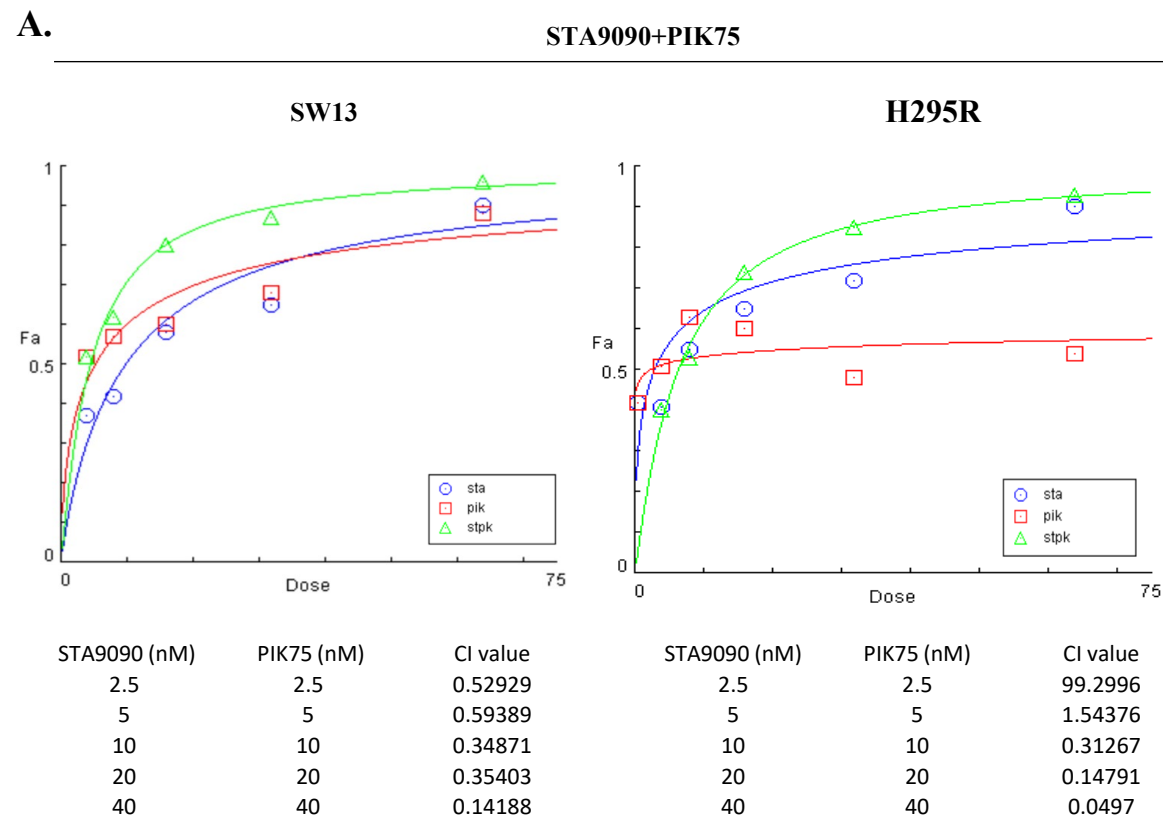
Supplementary Figure 3. Kaplan Meyer curve shows poor overall survival for adrenocortical carcinoma patients with high expression of HSP90B3P in comparison to group patients with lower expression of HSP90B3P.



Supplementary Figure 4. Kaplan Meyer curve shows poor disease-free survival for adrenocortical carcinoma patients with high expression of HSP90B3P, HSP90B2P and HSP90AB2P in comparison to group of patients with lower expression of HSP90B3P.

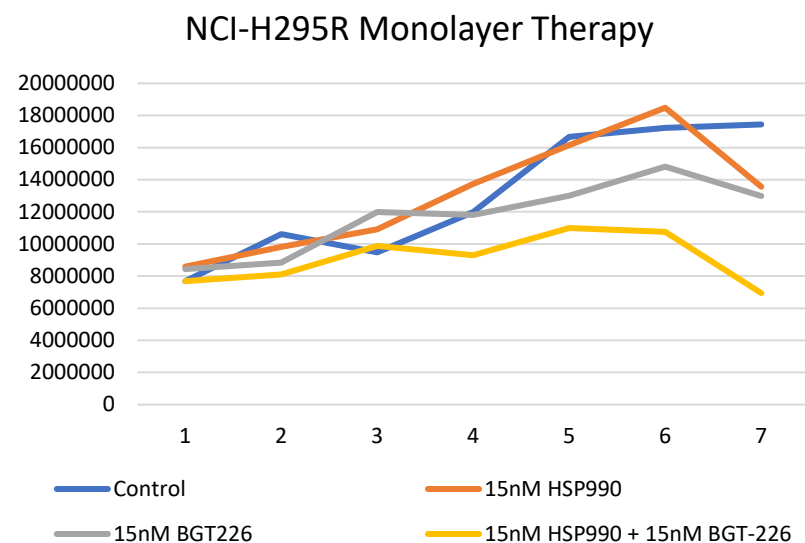


Supplementary Figure 5. Increased mRNA expression of HSP90B2P and AKT2 in **A.** Advanced stage III/IV (N=46) in comparison to stage I/II (N=31) adrenocortical carcinoma and **B.** Distant metastatic adrenocortical carcinoma (N= 15) group in comparison to the non-metastatic tumor (N=62) group in the TCGA dataset. Significance $P \leq 0.05$.

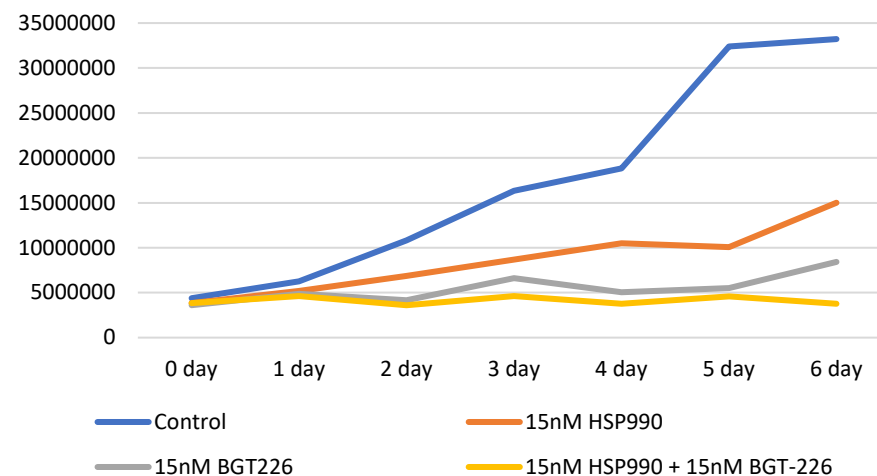


Supplementary Figure 6. Graphs representing HSP90 and PI3K inhibitors synergy in SW13 and H295R cells after treatment with **A.** STA9090 and PIK75 **B.** HSP990 and PIK75. Efficacy indicated by the combination index (CI) value was evaluated for five different dose combinations. CI < 1 indicated synergy; CI = 1 indicated an additive effect; and CI > 1, indicated an antagonistic effect

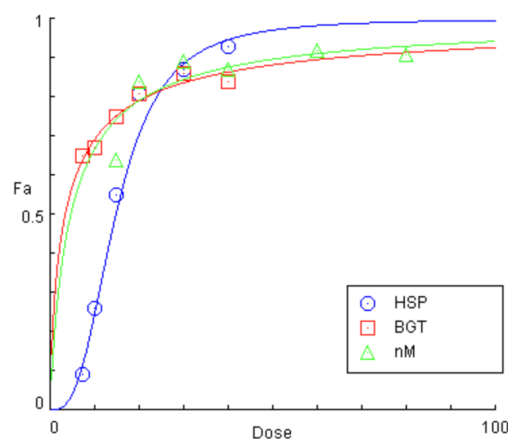
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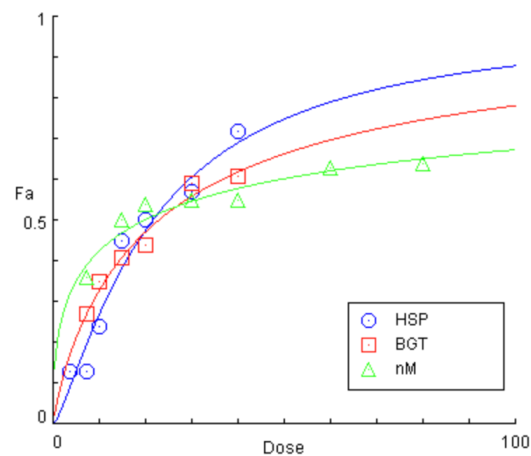
SW13 Monolayer Therapy



B.

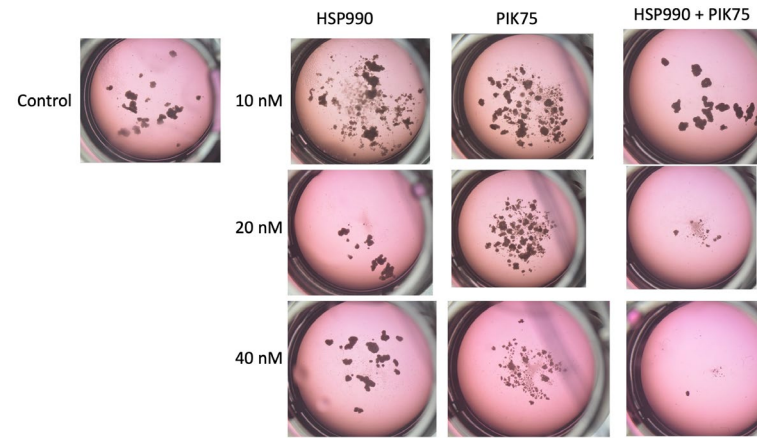
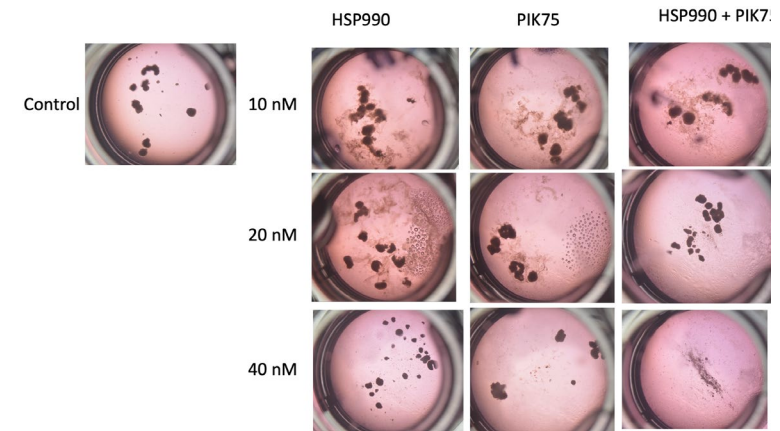
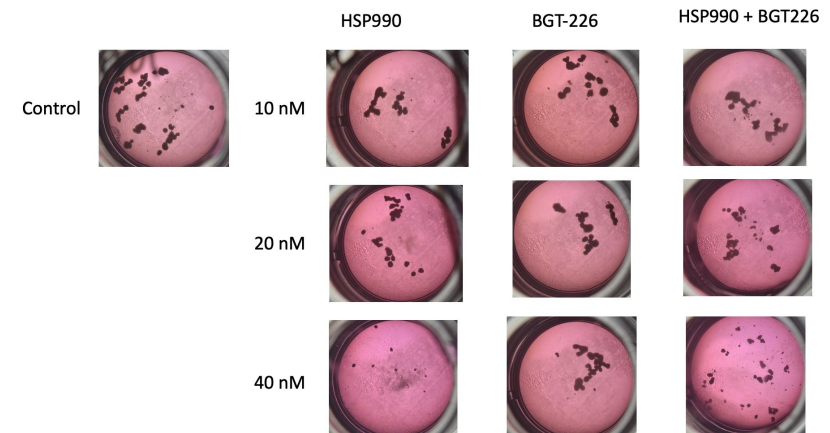
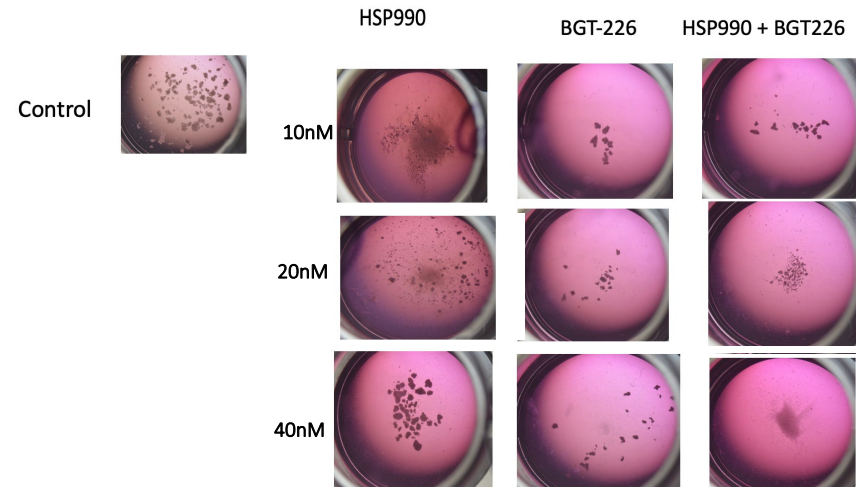


Total Dose	Fa	CI Value
15.0	0.64	1.44946
20.0	0.84	0.70471
30.0	0.89	0.76426
40.0	0.87	1.16849
60.0	0.92	1.21142
80.0	0.91	1.75340

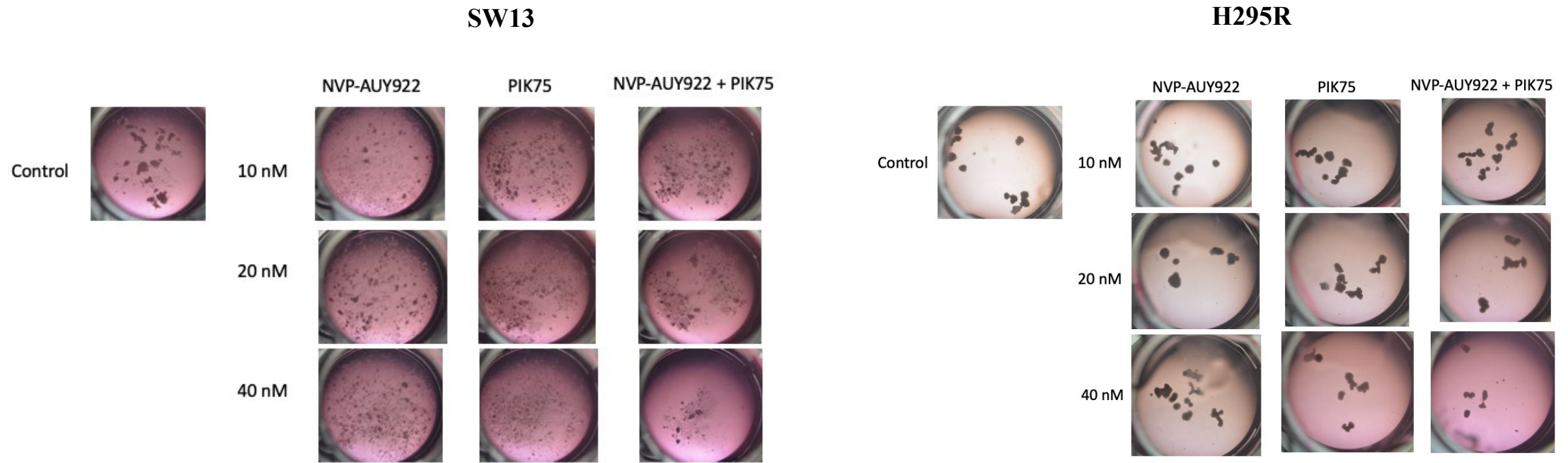


Total Dose	Fa	CI Value
7.5	0.36	0.59377
15.0	0.5	0.67919
20.0	0.54	0.77673
30.0	0.55	1.12114
40.0	0.55	1.49485
60.0	0.63	1.63826
80.0	0.64	2.09755

Supplementary Figure 7. Graphs representing proliferation curves of H295R and SW13 cells treated with HSP90 and PI3K drugs either as monotherapy or combination therapy of **A.** HSP990 and BGT226 **B.** Dose-effect curves indicating efficacy by the combination index (CI) value for five different dose combinations. CI < 1 indicated synergy; CI = 1 indicated an additive effect; and CI > 1, indicated an antagonistic effect

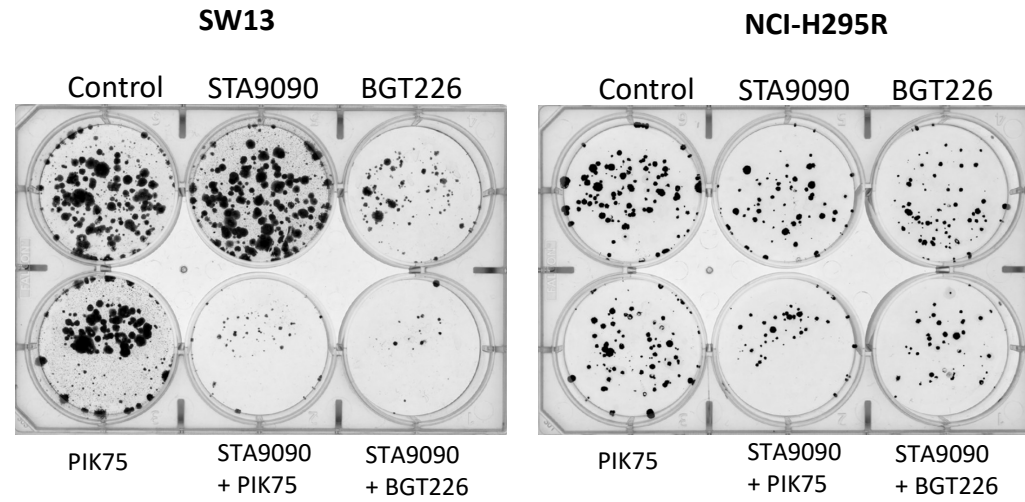
A.**SW13****H295R****B.**

Supplementary Figure 8. Monotherapy and combination therapy of demonstrating disintegration of 3-dimensional multicellular aggregates of cells **A.** HSP990 and PIK75 in the SW13 and H295R and **B.** HSP990 and BGT226 in the SW13 and H295R

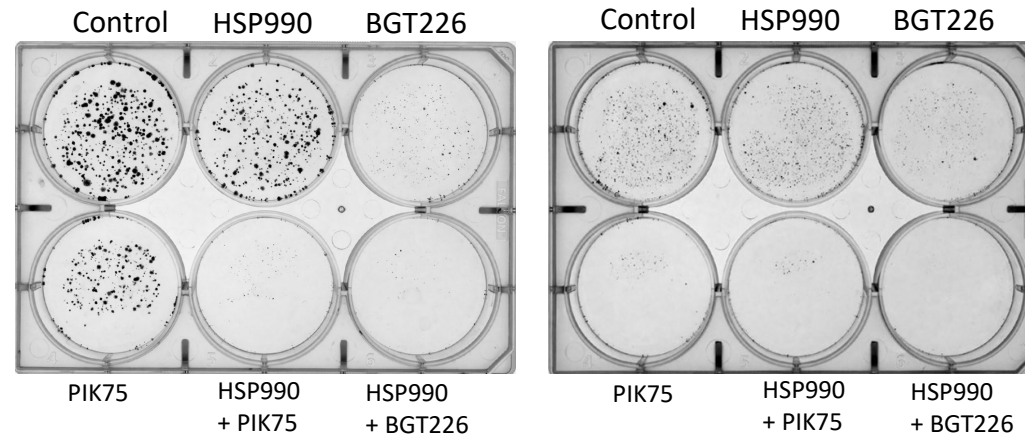


Supplementary Figure 9. Monotherapy and combination therapy of demonstrating disintegration of 3-dimensional multicellular aggregates of cells NVP-AUY922 and PIK75 in the SW13 and H295R

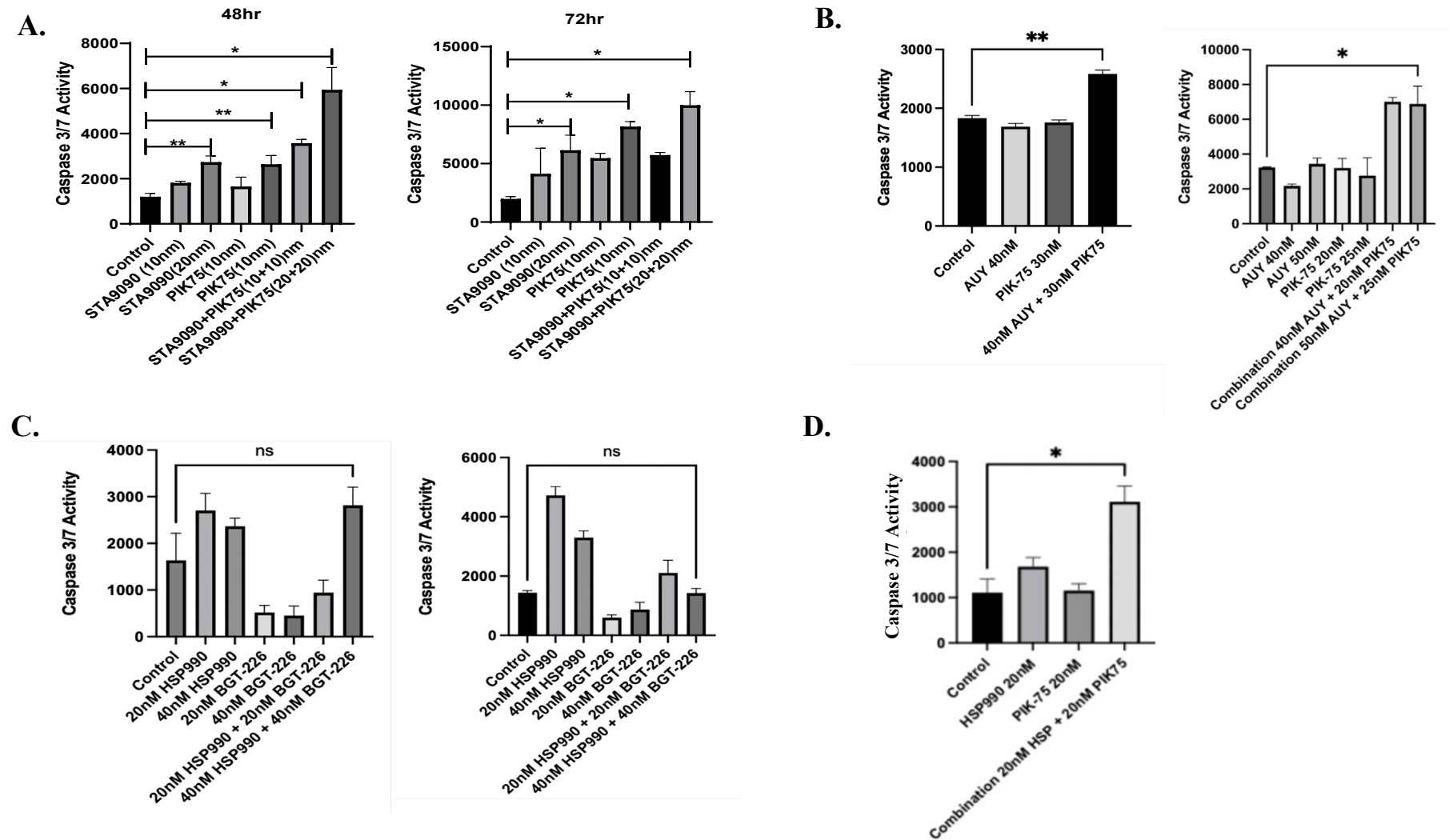
A.



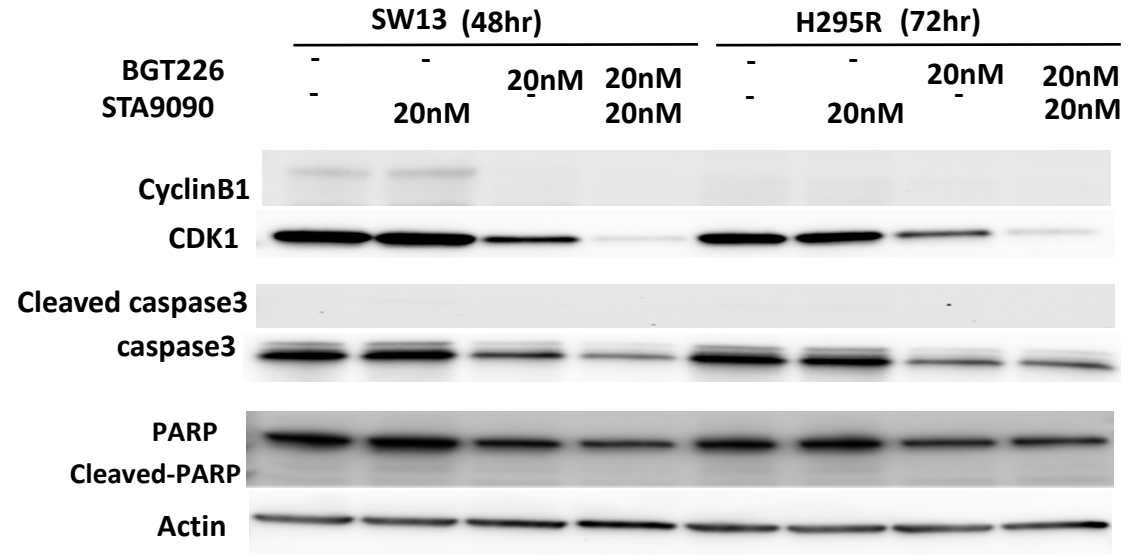
B.



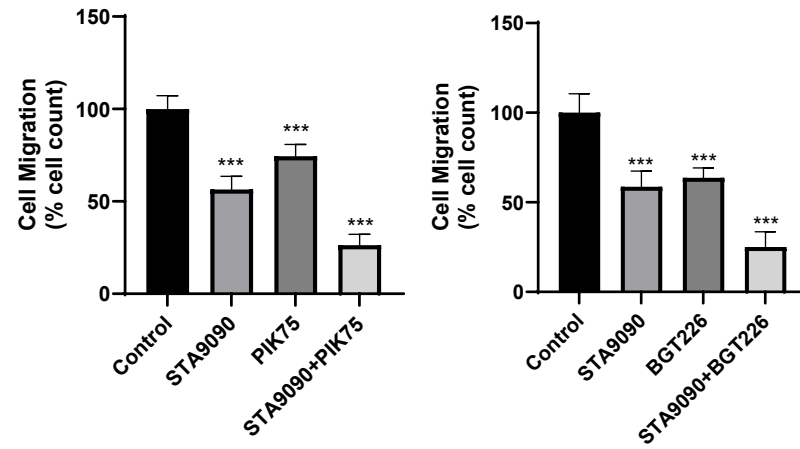
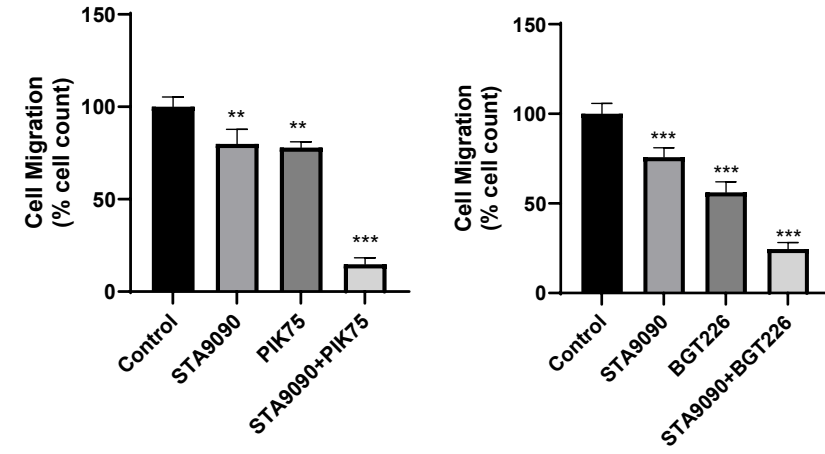
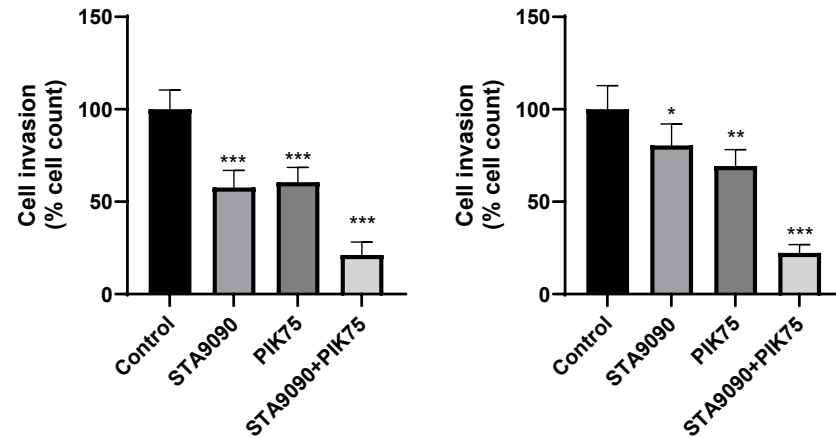
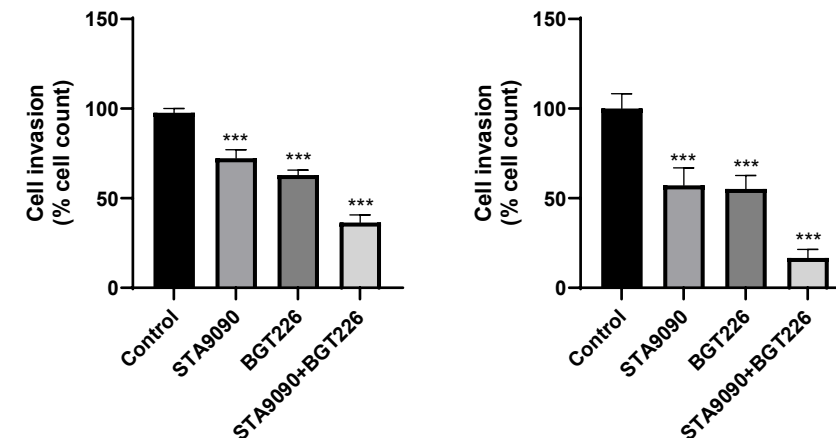
Supplementary Figure 10. Monotherapy and combination therapy demonstrating decrease in the colony formation of cells **A.** STA9090 and PIK75 or BGT226 in the SW13 and H295R **B.** STA9090 and PIK75 or BGT226 in the SW13 and H295R



Supplementary Figure 11. Caspase 3/7 activity after dose dependent manner treatment of the individual drugs and their combined treatment **A.** STA9090 and PIK75 **B.** AUY-922 and PIK75 **C.** HSP990 and PIK75, and **D.** HSP990 and BGT226. * $P \leq 0.05$; ** $P \leq 0.01$, ns, not significant.

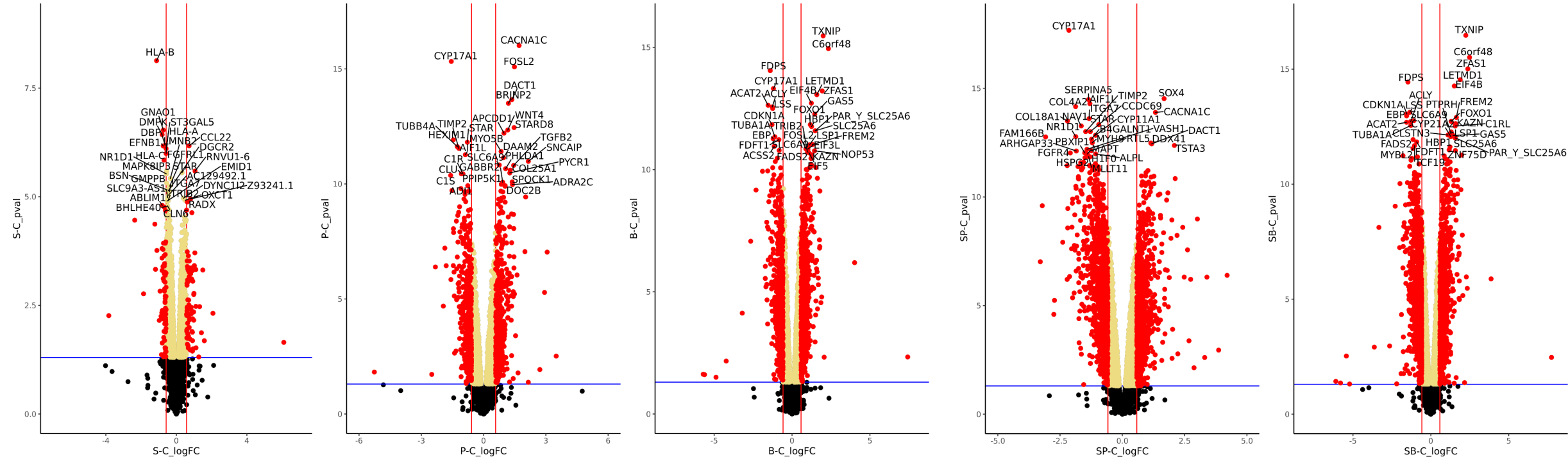


Supplementary Figure 12. Western blots showing inhibition of the protein expression of the cell cycle markers (cyclin B1 and cdk1) and apoptotic markers (Cleaved caspase3, caspase3, PARP, Cleaved-PARP) in SW13 and H295R after treatment of the inhibitors STA9090 and BGT226 for 48hrs and 72hrs respectively.

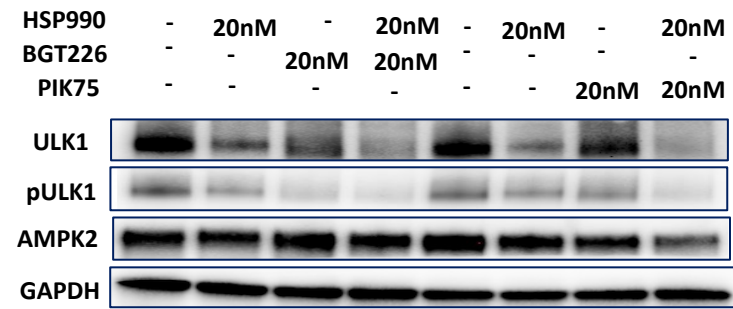
A.**B.****C.****D.**

Supplementary Figure 13. Quantitation of cell migration and invasion. Graphs show migration of **A.** SW13 and **B.** H295R cells, and invasion of **C.** SW13 and **D.** H295 cells, after treatment with STA9090 and PIK75 or BGT226 for 48 hours.

H295R

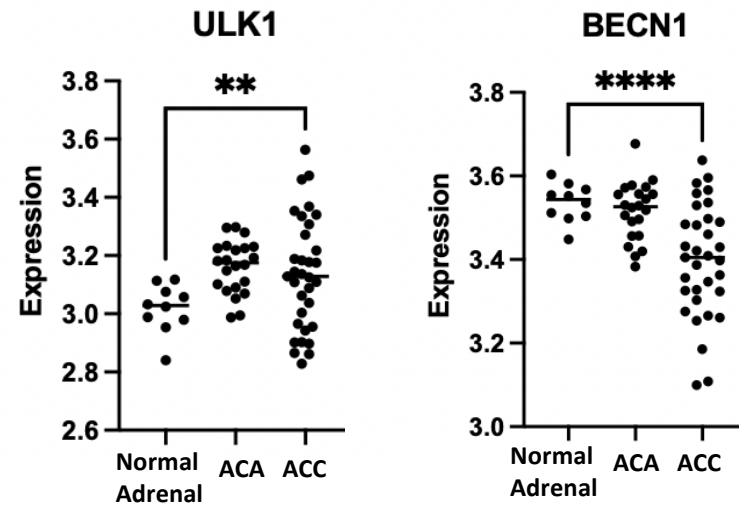


Supplementary Figure 14. RNA sequencing analysis in H295R cells A. Volcano plots for treatment groups (S; STA9090, P; PIK75, B; BGT226, SP; STA9090 and PIK75, SB; STA9090 and BGT226) vs C, Control. B. The log₂ FC indicates the mean expression level for each gene, and the horizontal blue line shows the cut-off value for significant genes P<0.05. Each dot represents one gene, the red dots to the left of log FC cutoff range (-1.3 to 1.3) represent down-regulated genes and red dots on the right of the log FC cutoff range represent up-regulated genes.

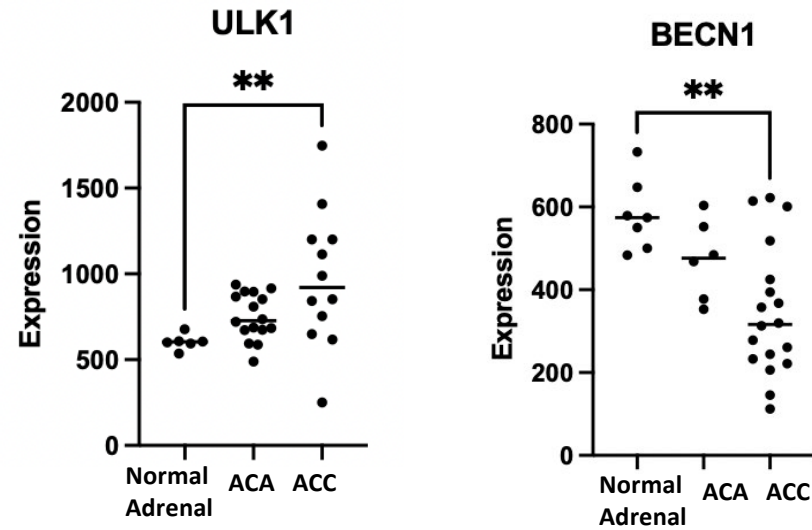


Supplementary Figure 15. Western blots showing inhibition of the protein expression of the autophagy markers (ULK1, pULK1, AMPK2, LAMP2, Beclin1, pBeclin1, GAPDH) in H295R after treatment of the inhibitors HSP990 and BGT226 or PIK75 for 72hrs.

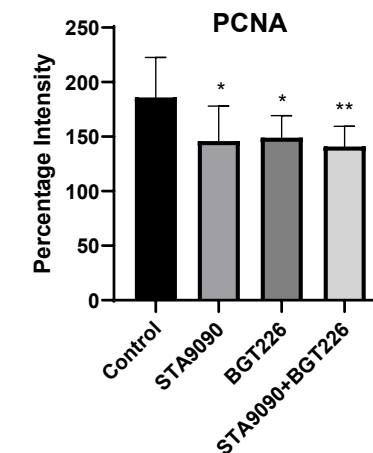
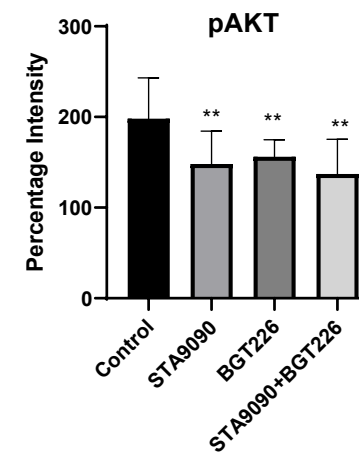
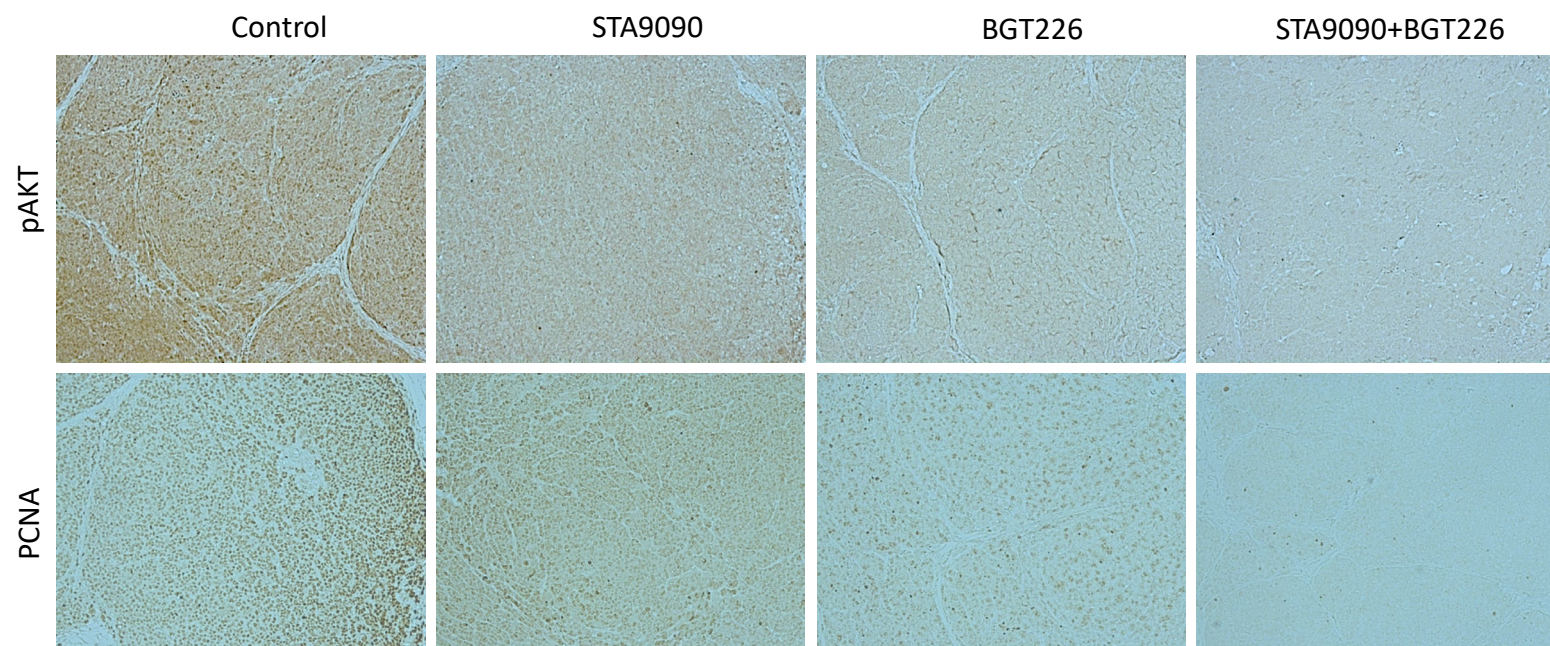
GSE12368



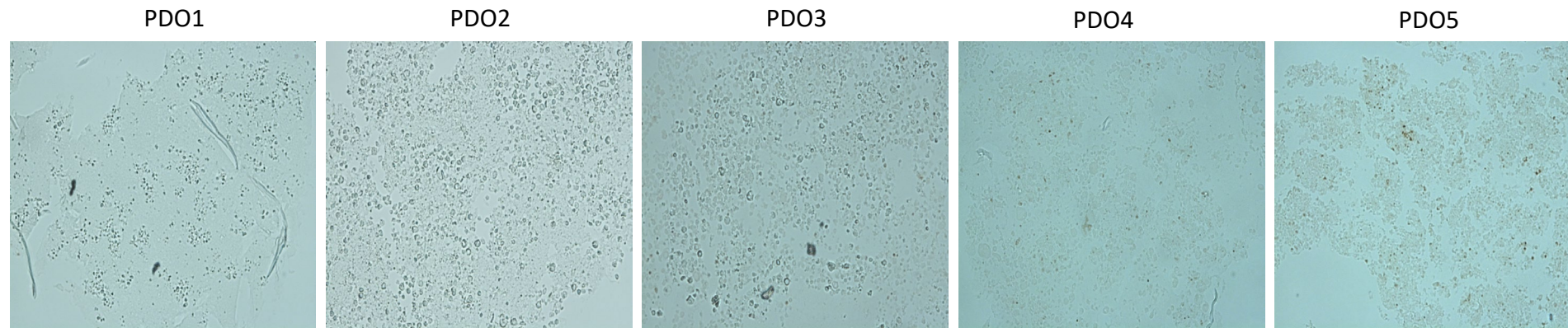
GSE75415



Supplementary Figure 16. Graphs showing mRNA expression for key members of autophagy (ULK1 and Beclin1) family in either of the GEO datasets GSE12368; Normal Adrenal (n=6), ACA (n= 16), ACC (n=12) GSE75415; Normal Adrenal (n=7), ACA (n= 5), ACC (n=18). ** $P \leq 0.01$, **** $P \leq 0.001$. ACA; Adrenocortical Adenoma. ACC; Adrenocortical Carcinoma.



Supplementary Figure 17. Immunohistochemistry showing expression of phospho-AKT (pAKT) and PCNA in mice tumor tissues in different treatment groups (Control, STA9090, BGT226, STA9090+BGT226) after 7 weeks. Graphs on the right show the percentage intensity of the targeted proteins in each of the treatment groups



Supplementary Figure 18. Immunohistochemistry showing expression of adrenocortical carcinoma marker steroidogenic factor (SF1) in five representative patient derived organoids (PDOs). Magnification 100x.