

Supplementary Information

Table S1. IC₅₀* of ganetespib, doxorubicin and etoposide in H82, GLC4 & H69 cells

	Ganetespib (nM)	Doxorubicin (nM)	Etoposide (nM)
H82	30.27	43.10	219.45
GLC4	20.47	37.9	245.20
H69	83.36	93.02	449.1

*IC₅₀s were determined by MTS assay as described in Materials and Methods.

Table S2. P53 and RB status

Cell lines	P53	RB
H82	p.T125T/c.375G>T ^{2,3}	p.V314 splice/c.940-2A>T ^{1,4}
GLC4	p.K132E/c.394A>G ^{2,3}	negative by IHC ⁵
N592*	?	?
H128	p.E62*/c.184G>T ³	p.R148fs/c.1252delA ¹
H146	p.P318fs*21/ c.953-971del 19 ¹	p.Q850*/c.2548C>T ¹
H69*	p.E171*/c.511G>T ¹	p.E748*/c.224G>T ¹

1. cancer.sanger.ac.uk/cancergenome/projects/cosmic/

2. PLoS One. 6(6):e21300, 2011

3. p53.iarc.fr/CellLines.aspx

4. broadinstitute.org/ccle/search

5. Virchows Arch 442:349-355, 2003

*H69 and N592 were derived from the same patient².

Figure. S1. A. Cell cycle analysis of N592 cells treated with doxorubicin (IC50=40nM) and genetesipib (IC50=20nM).

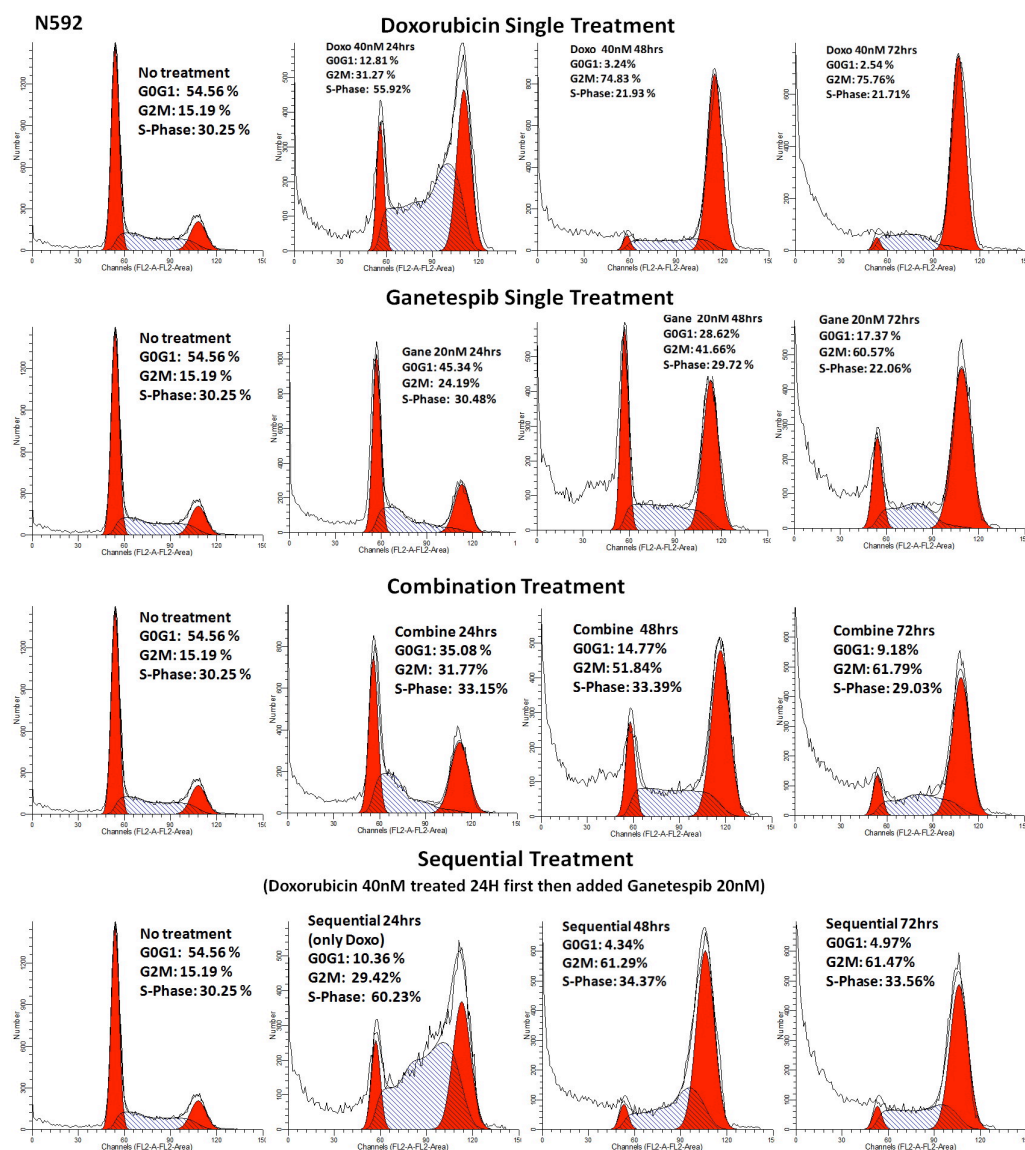


Figure S1. B. Cell cycle analysis of H69 treated with doxorubicin (IC50= 120nM) and ganetespib (IC50=50nM).

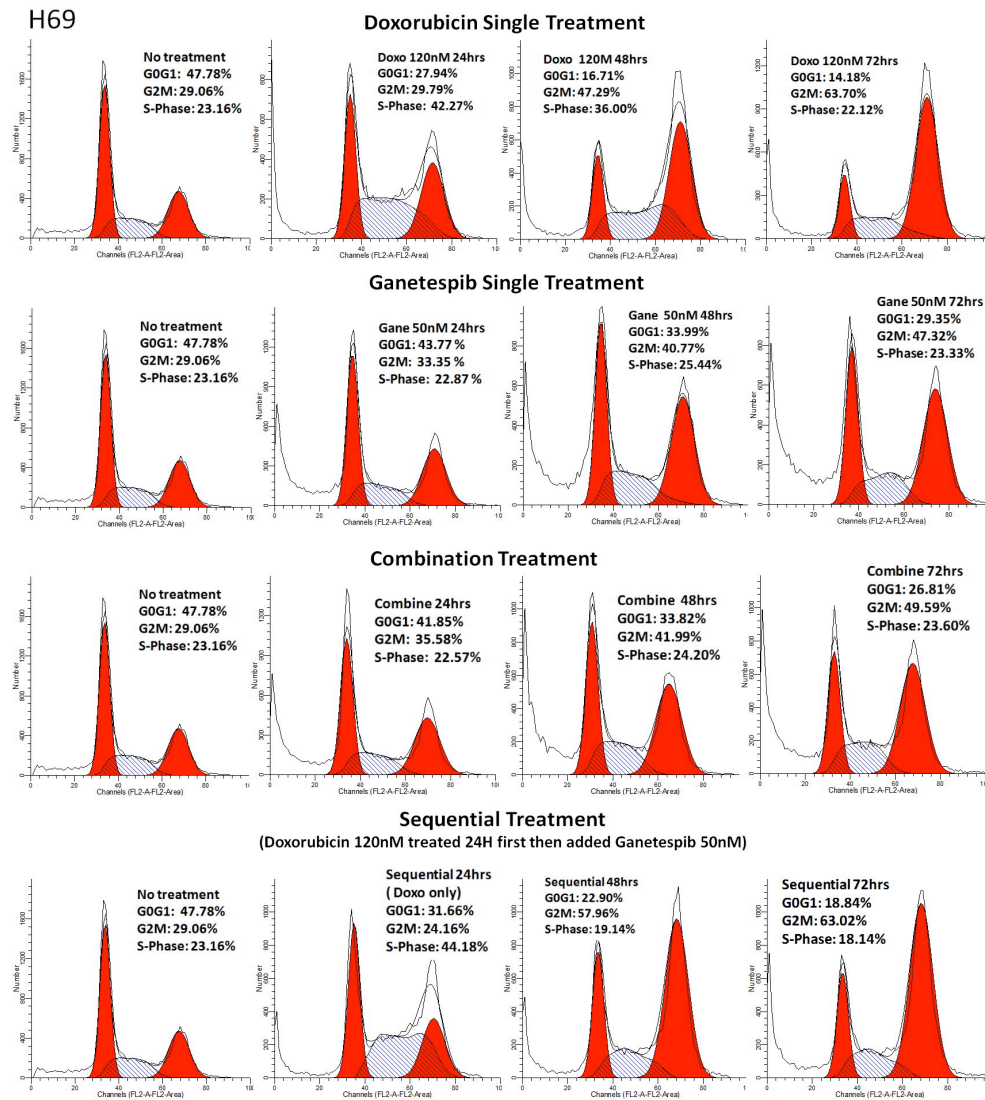


Figure S2. MTS assay on the combination index (CI) of doxorubicin (Doxo) + ganetespib (Gane), and etoposide (Etop) + ganetespib (Gane) in H69 cells. Combination index (CI) was calculated using Calcsyn algorithm. Each number (1 to 7) in the graph represents drug concentrations from top to bottom in the table. Number 4 is IC50 of each drug.

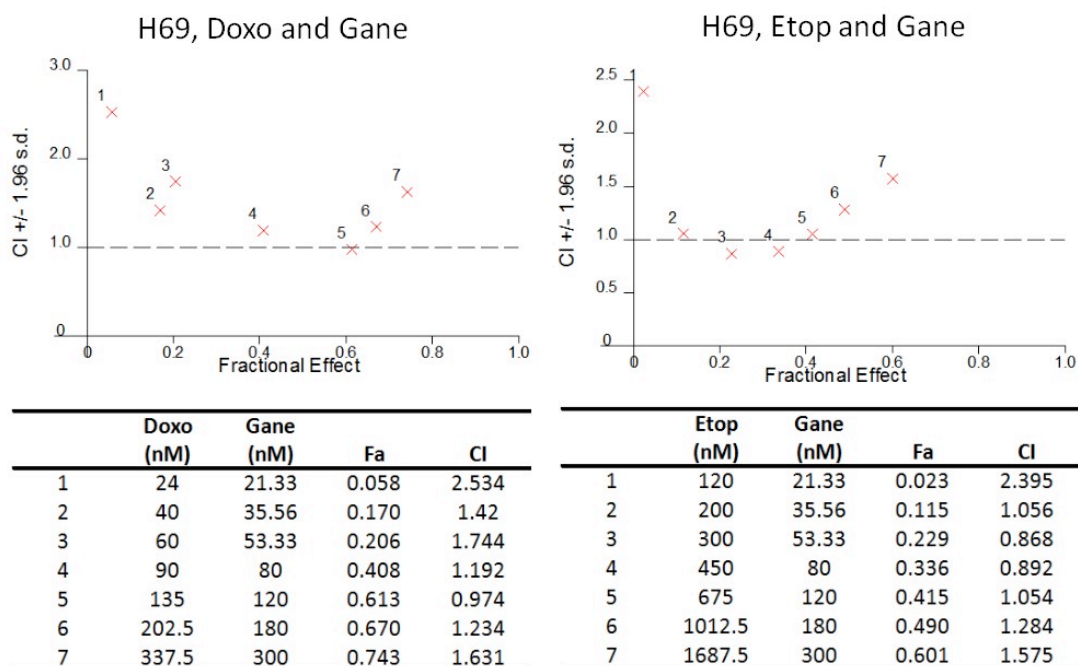


Figure S3. A. Unsupervised hierarchical clustering of cancer-associated protein expressions and phosphorylations profiled by RPPA. Horizontal axis illustrates clustering analysis of 81 proteins and phospho-proteins in H82 cells treated with different drug regimens (vertical axis). Doxo, doxorubicin; Gane, ganetespib; Etop, etoposide; Comb GR1, doxorubicin + ganetespib; Comb GR2, etoposide + ganetespib. **B.** Pathway analysis of protein networks affected by doxorubicin + ganetespib combination treatment. Each of the node shapes denotes the function of the interacting proteins. The connectivity map was created from available published data using the Ingenuity Interactive Pathway (IPA) Analysis which compiled data from interactions validated in multiple model organisms. Red and green nodes represent proteins or phospho-proteins that were either upregulated or downregulated respectively as defined in Table 2. White nodes are proteins that exhibit direct connectivity with the networks identified by IPA. These include Caspase 3 (CASP3) and RIP1 (RIPK1) which were confirmed by western blot analysis (Figure 3A).

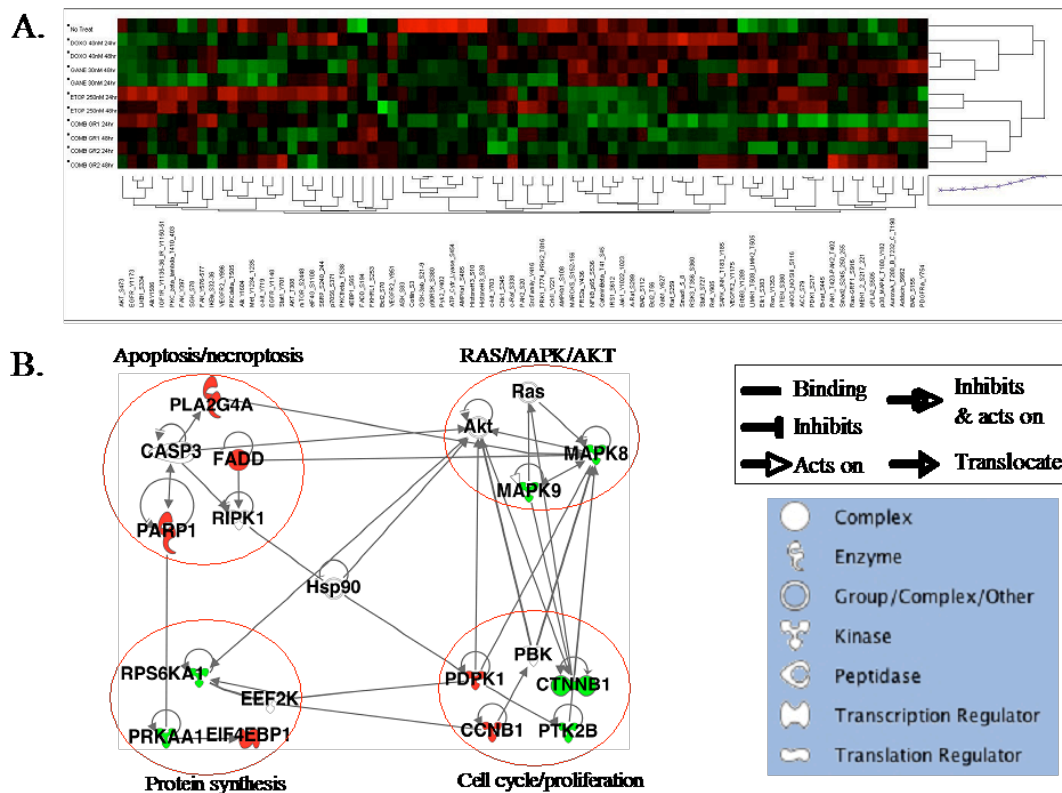


Figure S4. Western blot analysis of Rip1 in GLC4 cells treated with doxorubicin, ganetespib, and the combinations at the indicated concentrations for 24 and 48 hours respectively. Note that ganetespib is more efficient in reducing RIP expression than 17-AAG. Comb G+D, ganetespib and doxorubicin combination; Comb 17+D, 17-AAG and doxorubicin combination.

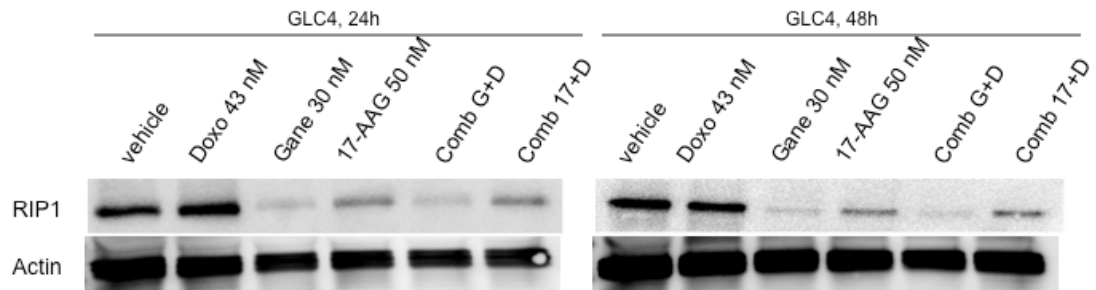


Figure S5. TUNEL stain of GLC4 cells. a. Negative control siRNA; b. 40nM doxorubicin; c. 10nM RIP1 siRNA; d. 40nM Doxorubicin and 10nM RIP1 siRNA combination after 72h exposure. 400X magnification. Note that as a transfection reagent control, lipofectamine was added in doxorubicin treatment group.

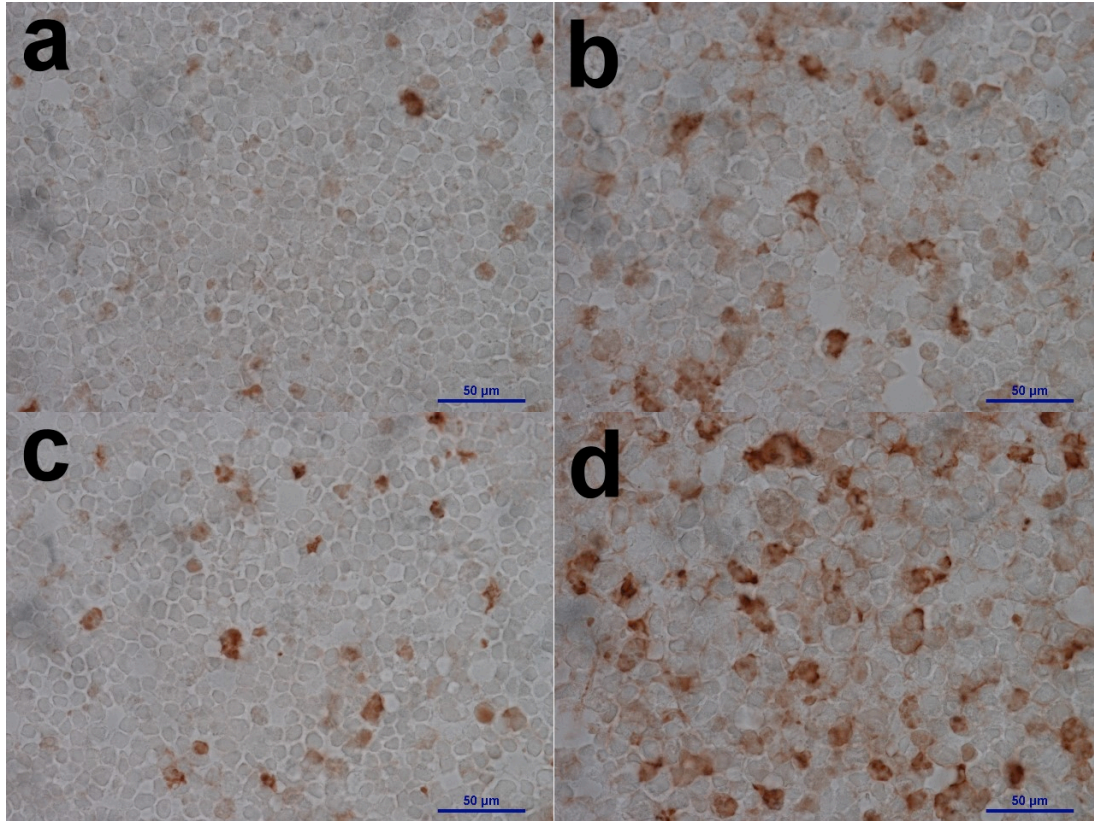


Figure S6. Mouse xenograft study of GLC4 cells. p-value was calculated by one-way ANOVA at day 20 after drug treatment. p values were significant between any two group comparisons except for the doxorubicin and ganetespiib comparison. %T/C value was calculated as illustrated in Fig. 4A. Bars indicate standard errors. Drug doses and schedules are indicated in the graph.

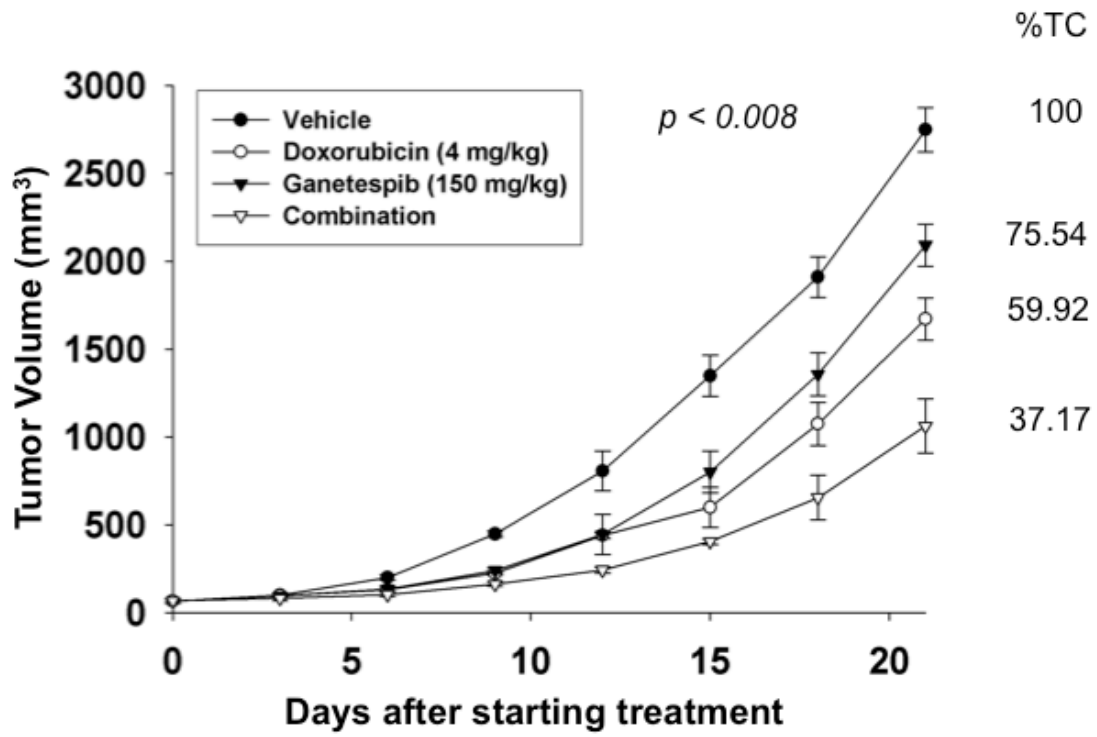


Figure S7. Representative images of the growth of H82 xenografts on day 20 after drug treatment. From left to right: vehicle, doxorubicin 4mg/kg treated every other day, ganetespiib 150mg/kg treated every week, and combination treatment. Arrow marks the tumor site.

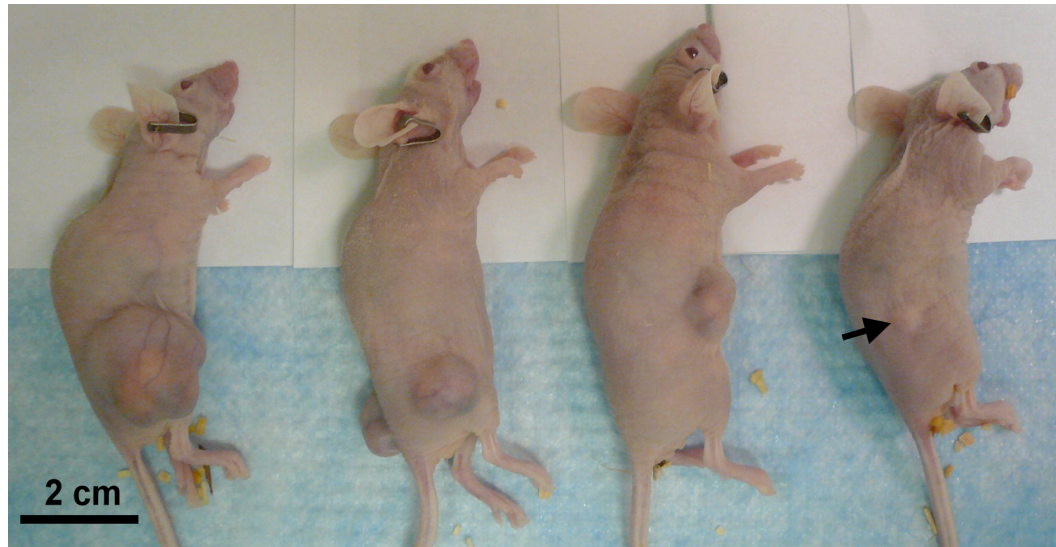


Figure S8. Western blot analyses of γ -H2AX in GLC4 and H82 cells treated with ganetespib, 17-AAG, doxorubicin or the combinations at the indicated concentrations. Note that γ H2AX was induced at 24 hrs and 48 hrs in H82 and GLC4 cells respectively after doxorubicin treatment, whereas no significant induction of γ H2AX was observed upon ganetespib or 17-AAG treatments at either time point. Comb G+D, ganetespib and doxorubicin combination; Comb 17+D, 17-AAG and doxorubicin combination.

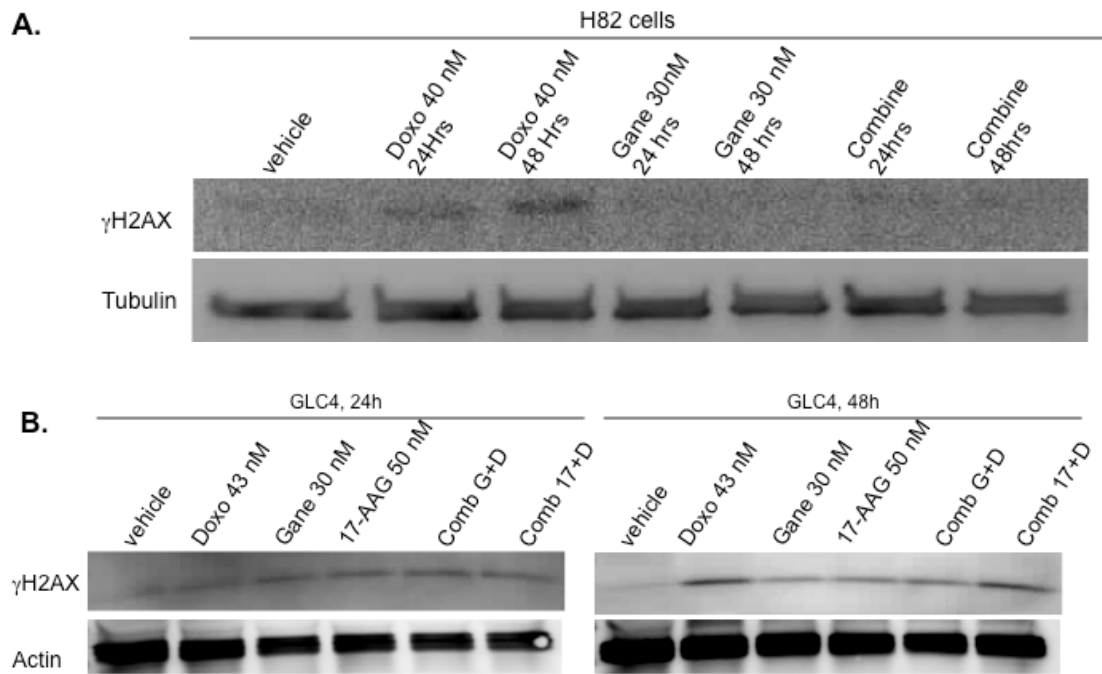


Figure S9. A. Western blot analysis of I κ B-a expression in GLC4 cells treated with different regimens of drugs as indicated. B. Western blot analysis of I κ B-a and RIP1 expression in GLC4 cells 72hrs after siRNA transfection as indicated. No Txt: no treatment.

