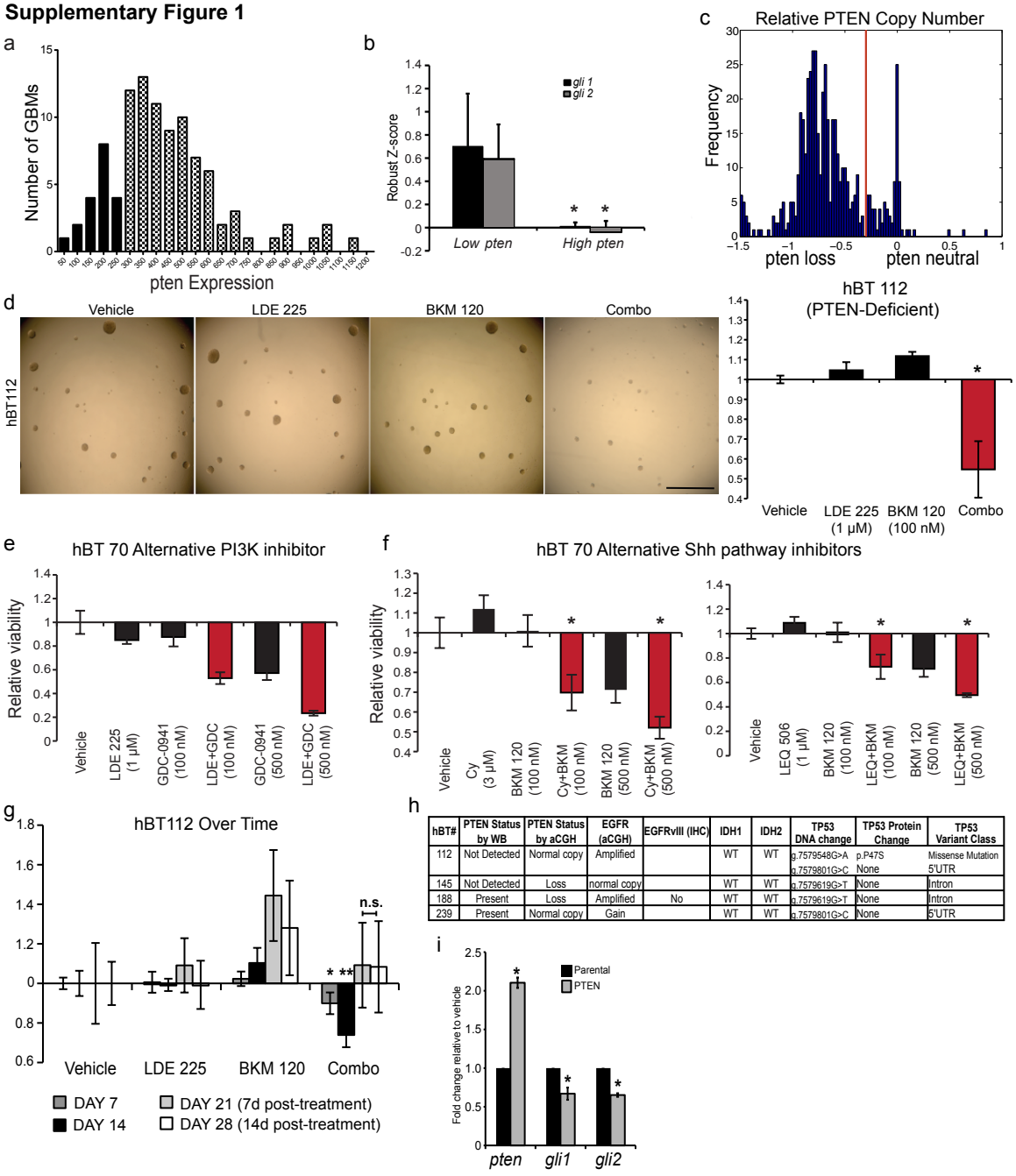
Supplementary Figure 1



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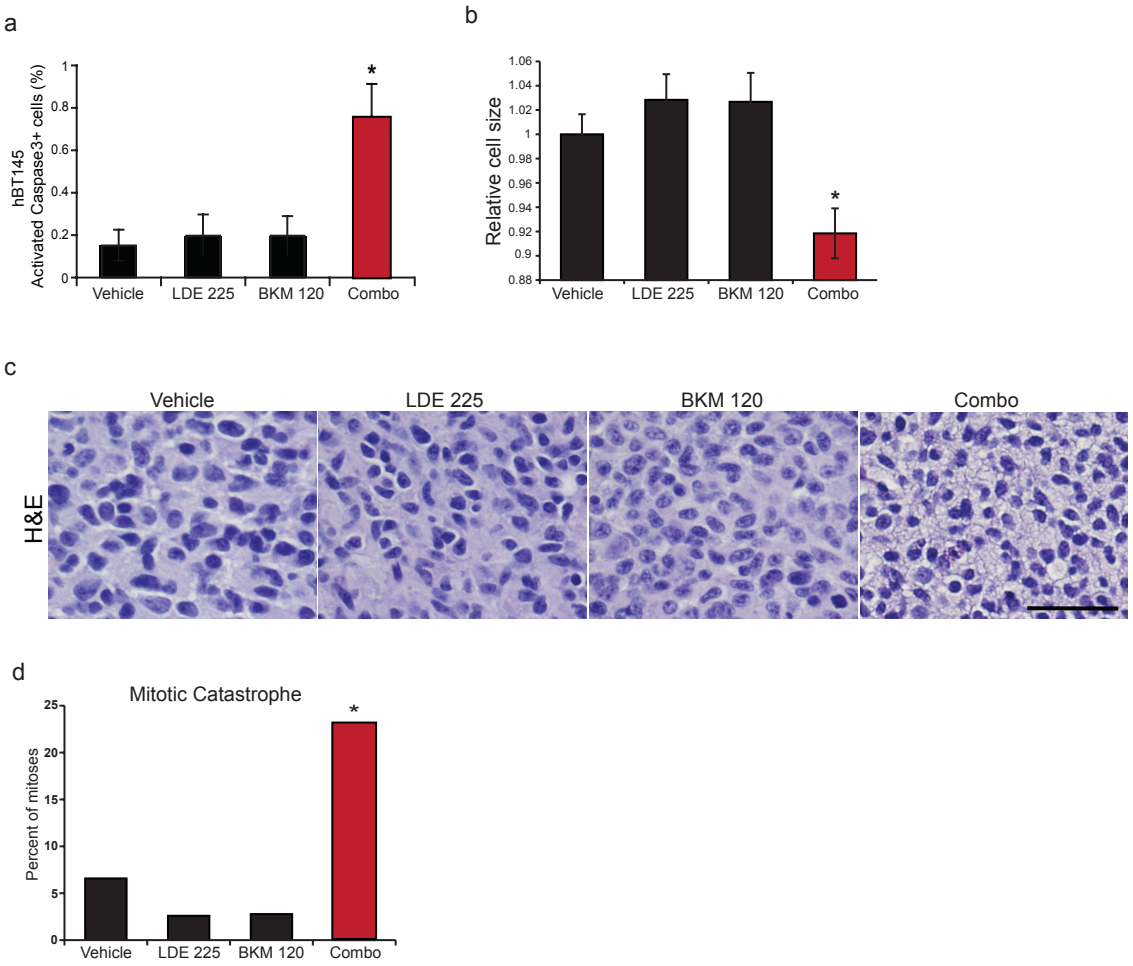
hBT#	PTEN Status by WB	PTEN Status by aCGH	EGFR (aCGH)	EGFRvIII (IHC)	IDH1	IDH2	TP53 DNA change	TP53 Protein Change	TP53 Variant Class
112	Not Detected	Normal copy	Amplified		WT	WT	g.7579548G>A g.7579801G>C	p.P47S None	Missense Mutation 5'UTR
145	Not Detected	Loss	normal copy		WT	WT	g.7579619G>T	None	Intron
188	Present	Loss	Amplified	No	WT	WT	g.7579619G>T	None	Intron
239	Present	Normal copy	Gain		WT	WT	g.7579801G>C	None	5'UTR

(**a**) Histogram of *pten* expression from first database analyzed [7]. Black bars indicate samples considered to express low *pten* (22% of tumors); crosshatched bars indicate high *pten* samples. (**b**) GBMs with decreased *pten* exhibit increased activation of the Shh signaling pathway; robust z-scores for *gli1 and gli2* are higher in tumors expressing low *pten* compared with others (Supplementary Figure 1a), (*) p = 0.005. (**c**) Copy number analysis of *pten* status (Broad database, TCGA [8]). Red line indicates cut-off used for loss of *pten*

(–0.3 threshold represents minimum in copy number distribution). (**d**) hBT112 neurospheres were imaged and viability was quantified by Trypan Blue exclusion (n = 3). Error bars: +/– SEM. (*) F = 12, DFn = 1, DFd = 20, p = 0.0025 by two-way ANOVA factorial interaction. Scale bar = 1 mm. (**e**) Combination treatment of alternative PI3K inhibitor GDC-0941 with LDE225 reduces cell viability in hBT70 monolayer (n = 3). Error bars: +/– SEM. F = 2.72, DFn = 2, DFd = 12, p = 0.1065 by two-way ANOVA factorial interaction. (**f**) Combination treatment of BKM120 with Smo-inhibitors, Cyclopamine, or LEQ506 reduces cell viability in hBT70 neurospheres (n = 3). Error bars: +/– SEM. (*) F = 5.60, DFn = 2, DFd = 2, p = 0.0192 for Cyclopamine and (*) F = 4.99, DFn = 2, DFd = 12, p = 0.0265 for LEQ506 for interaction with BKM120, by two-way ANOVA factorial interaction. (**g**) hBT112 neurospheres were assayed for viability (n = 3). Error bars: +/– SEM, (*) $p < 0.05$ and (**) $p < 0.01$ by one-way ANOVA. (**h**) Table of mutation status of hBT112, 145, 188 and 239 GBMs. Absence of PTEN protein is the only known factor common to lines responsive to combination therapy. (**i**) Acute overexpression of PTEN in hBT112 cells decreases expression of *gli1* and *gli2* (*, p = .005 for *gli1* and p = .00006 for *gli2* by Student t-test, mRNA normalized to *gapdh*), (n = 3). Results shown are normalized to no virus.

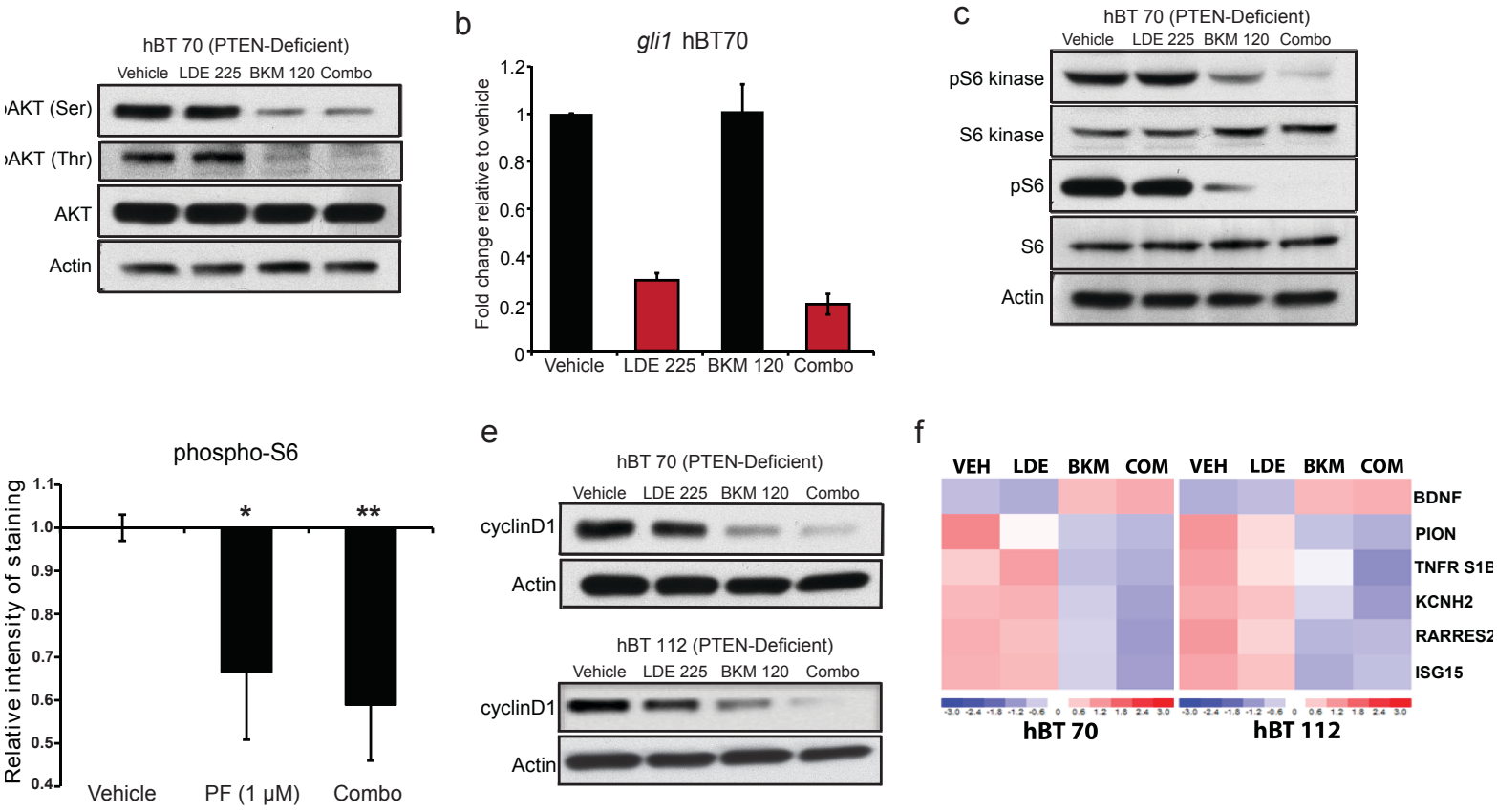
Supplementary Figure 2



(**a**) hBT145 cells were treated with vehicle, LDE225 (1 μM), BKM (100 nM) or combination therapy. Apoptotic index measured by activated caspase-3 is increased with combination therapy (*n* = 3). Error bars: +/– SEM, (*) $p < 0.01$ by chi-square analysis. (**b**) Combination treatment leads to a significant reduction of cell size as measured by M-phase cells, normalized to cells treated with vehicle. Data represent means from ≥ 50 cells per condition (*n* = 3). Error bars: +/– SEM, (*) $p = 0.002$ by t-test. (**c**) Hematoxylin/Eosin staining of hBT112 tumor xenografts at end treatment shows decreased cell size and increased pyknotic nuclei with combination therapy. Scale bar = 50 μM. (**d**) Combination therapy leads to increased deaths shortly post-mitosis; a cell divides and both daughter cells rapidly die (75% die within one hour after division and 90% die within two hours after division). All mitoses were analyzed; the proportion wherein both daughters rapidly die is shown. Data represent means from ≥ 52 cells per

condition, from three independent real time imaging experiments, (*) $p < 0.05$ by chi-square analysis.

ıpplementary Figure 3



(**a**) Immunoblots of phosphorylated and total AKT in hBT70 cells treated for 24 hours with LDE225 (1 μM), BKM120 (500 nM) or combination, Actin: loading control ($n = 3$). (**b**) Real-Time RT PCR *for gli1* mRNA normalized to *gapdh* ($n = 2$). (**c**) Immunoblots of phosphorylated and total S6K and S6 proteins from hBT70. Actin: loading control. (**d**) Phosphorylated S6 decreases in hBT112 cells after treatment with PF-4708671, as shown by integrated intensity of staining over number of cells counted. The effect is similar to that seen with combination therapy. Data represent means of ≥ 2000 cells ($n = 3$). Error bars: +/– SEM, (*) $p = 0.017$, (**) $p = 0.0008$ by z-test, compared to one. (**e**) Immunoblot of cyclinD1

from treated hBT70 and hBT112 cells. Actin: loading control. (f) hBT70 and hBT112 cells were treated with vehicle, LDE225 (1 μM), BKM120 (500 nM), or combination. mRNA expression was determined with Affymetrix Human Genome U133A 2.0 chips. Results are shown as heat map generated with unsupervised clustering. Data represent means of two independent experiments per line. These genes are significantly altered in combination therapy in both GBM lines. The full data set is in Table1.

Supplementary Table 1

Affymetrix data for hBT70 and hBT112 cells treated with vehicle control, LDE225 (1 μM), BKM120 (500 nM), or the combination of both for 5 d.