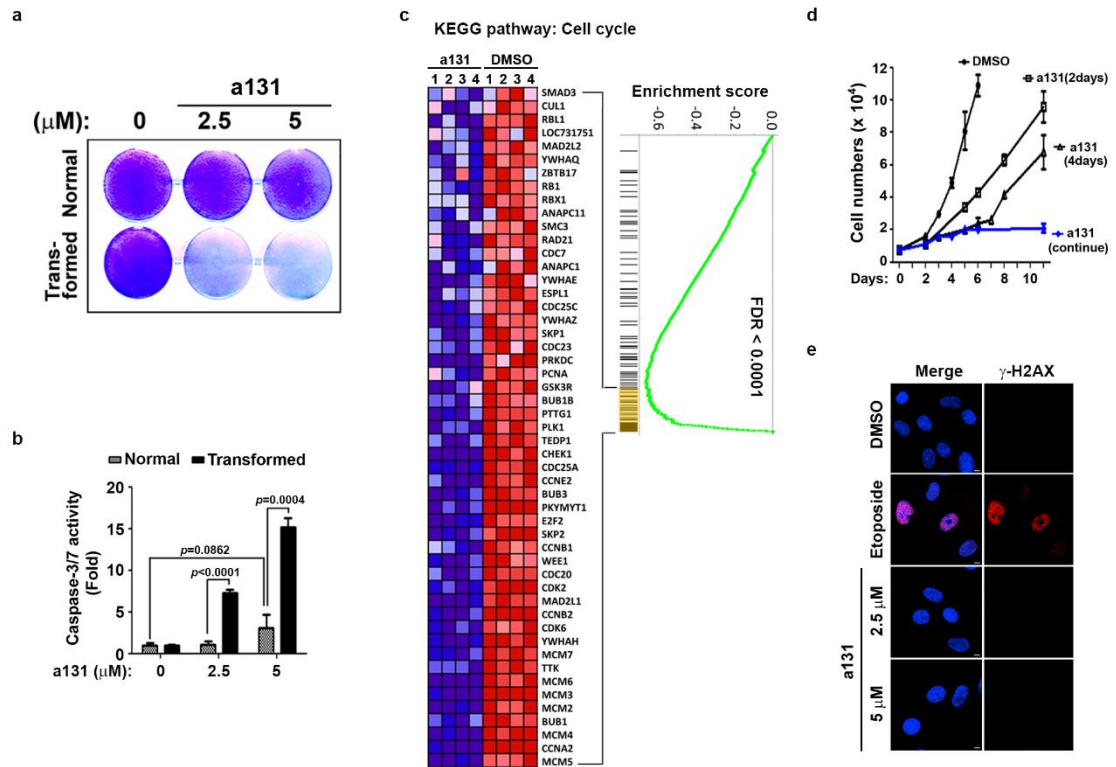




Supplementary Figure 1 | Selective killing effects of a131 in transformed BJ cells without inducing genotoxic stress.

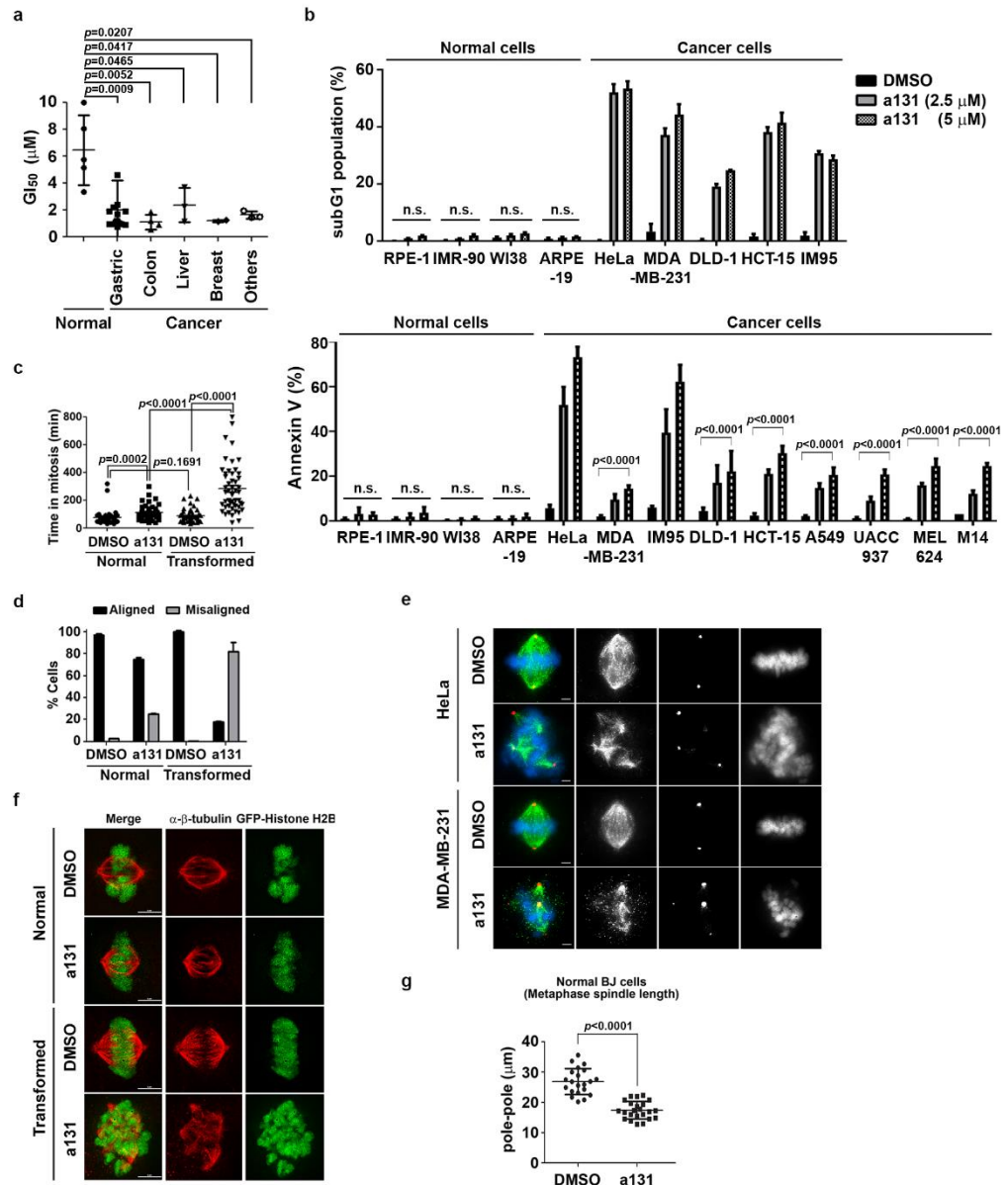
(a) Crystal violet assay of isogenic normal and transformed BJ cells treated with a131 for 72 h.

(b) Normal and transformed BJ cells were treated for 48h with the indicated concentrations of a131. Selective increase in combined activity of caspase-3/7 in transformed BJ cells by a131 treatment is shown. Mean values with $\pm$ S.D. are shown (n=3). Two-tailed unpaired t tests were performed to determine statistical significance.

(c) GSEA enrichment plot and heatmap of KEGG 'cell cycle' pathway genes in BJ cells treated with a131 for 24 h, compared to controls. The enrichment graph plots the enrichment scores for each gene (represented as bars), which are rank-ordered by their signal-to-noise metric between the DMSO control and a131 treated samples. Genes contributing to core enrichment of the pathway are highlighted in yellow. The per-sample expression profiles of these genes are depicted in the heatmap using an intensity-based, row-normalized color scale from blue to red, with blue indicating lower expression.

(d) Normal BJ cells were synchronized at the G₁ phase by serum starvation (0.1% FBS) for 2 days. Subsequently, the cells were synchronously released in fresh media with 10% FBS and then treated with 5 μ M a131 for 2, 4 or 11 days. After 2 or 4 days, a131 was removed and cell proliferation continued in fresh media for up to 11 days. The total number of cells at various time points were calculated using automated cell counter (SCEPTOR, Merck) and mean values with $\pm$ S.D. are shown (n>6).

(e) Normal BJ cells were treated with a131 at 2.5 and 5 μ M, etoposide at 100 μ M or DMSO vehicle control for 48 h and subjected to immunofluorescence analysis using anti- γ -Histone H2AX (γ -H2AX) antibody and DAPI. Note that while etoposide treatment markedly induced DNA-damage visualized by staining for γ -H2AX, a131 treatment did not do so as similar to DMSO treatment. Scale bar: 5 μ m.



Supplementary Figure 2 | Selective killing effects of a131 by inducing centrosome de-clustering in transformed BJ cells and various cancer cell lines.

(a) Human normal and cancer cell lines were treated with a131 at a range of different concentrations (from 0.1 μ M to 40 μ M) for 72 h in triplicate and cell viability was determined by MTT assay. Mean concentration values for a131 to achieve 50% growth inhibition (GI_{50}) in each groups of normal and different cancer cell lines by tissue type are plotted. Mean values with $\pm$ S.D. are shown. Two-tailed unpaired t test was performed to determine the statistical significance.

(b) Indicated cancer and normal cell lines were treated with a131 at 2.5 or 5 μ M for 48 h. Cells were collected and stained with PI (top) or Annexin V (bottom) and subjected to FACS analysis for subG1 (<2N) population (top) and Annexin V positive population (bottom) as indication of cell death via apoptosis. Mean values with $\pm$ S.D. are shown ($n > 3$). Two-tailed unpaired t test was performed to determine the statistical significance. n.s.: not significant

(c, d) Normal and transformed BJ cells stably expressing GFP-histone H2B were treated with a131 at 2.5 μ M or DMSO vehicle control for 8 h and subjected to time-lapse live-cell imaging for 24 h with 5 min intervals.

(c) The duration of mitotic progression after nuclear envelop breakdown until completion of cell division in each randomly selected cell is presented ($n > 50$ per condition). Mean values with $\pm$ S.D. are also shown from triplicated experiments. Two-tailed unpaired t test was performed to determine the statistical significance.

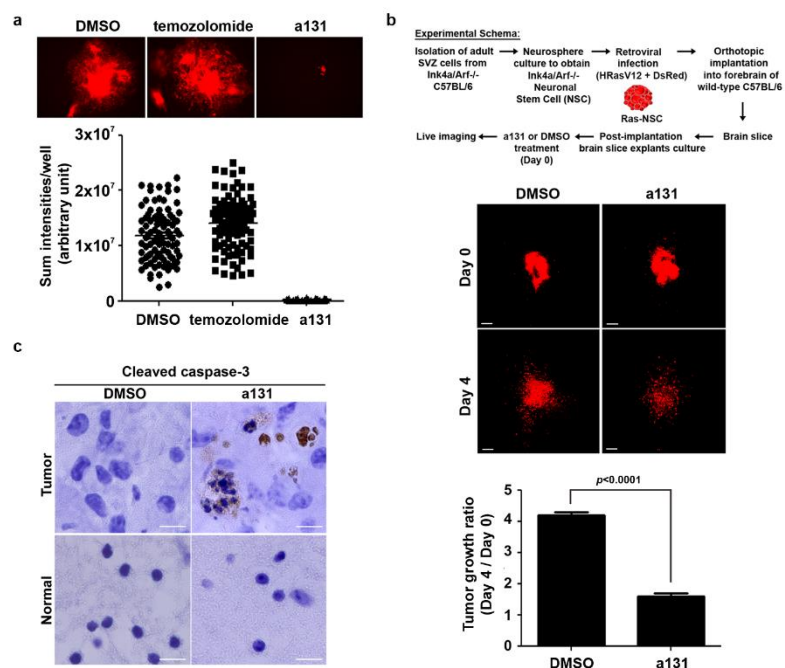
(d) Subsequently, cells were fixed in PFA and subjected to immunofluorescence analysis using antibodies against β -tubulin and γ -tubulin. Images were obtained using 3D-SIM super resolution microscopy and representative images are shown in Fig. 1g. Quantification of cells ($n > 50$ per condition) with misaligned chromosomes. Mean values with $\pm$ S.D. are shown from triplicated experiments.

(e) Indicated cancer cell lines were treated with a131 at 2.5 μ M or DMSO vehicle control for 12 h and subjected to immunofluorescence analysis as in d and cells were counterstained with DAPI. Images were obtained using 3D-SIM super resolution microscopy. Scale bar: 5 μ m.

(f, g) Normal and transformed BJ cells stably expressing GFP-histone H2B were treated with a131 at 2.5 μ M for 24 h. Subsequently, cells were fixed in PFA and subjected to immunofluorescence analysis using antibody against β -tubulin. Images were obtained using 3D-SIM super resolution microscopy.

(f) a131 treatment caused massively misaligned chromosomes with multipolar spindles in transformed BJ cells (bottom panels), but not in normal BJ cells (top panels). Scale bar: 5 μ m.

(g) Quantification of metaphase spindle length (pole-pole distance) (n>20 cells) in normal BJ cells. Mean values with $\pm$ S.D. are shown from triplicated experiments. Two-tailed unpaired t test was performed to determine the statistical significance.

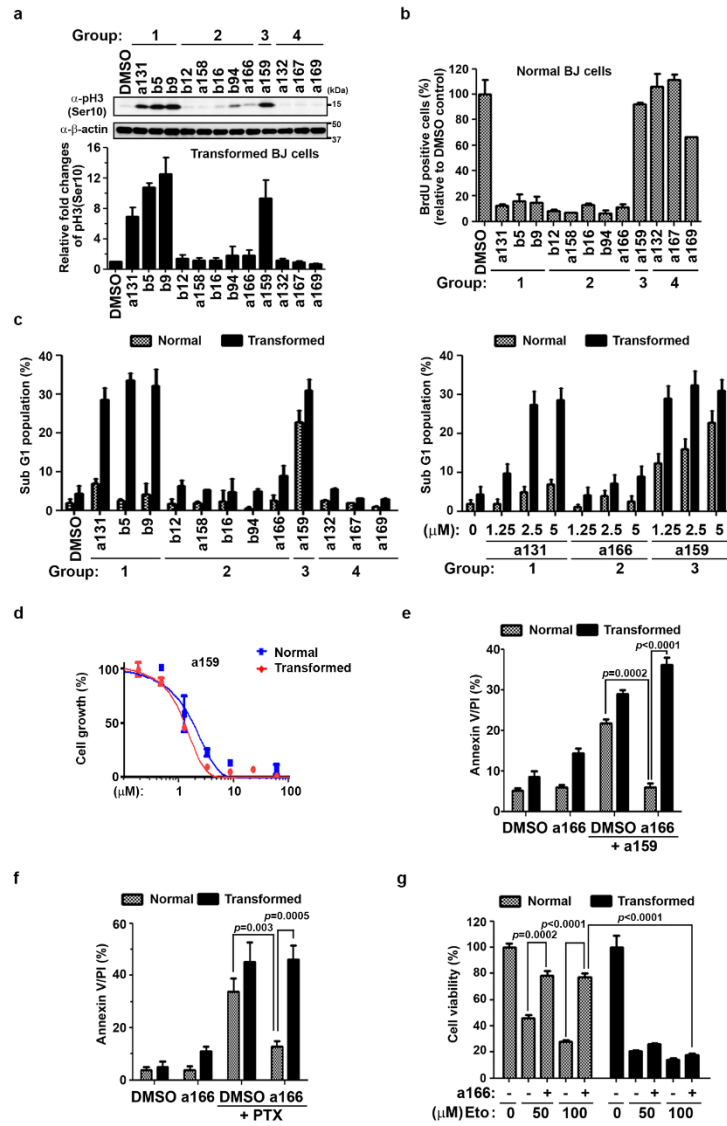


Supplementary Figure 3 | a131 suppresses growth of Ras-driven glioma initiating cells (GIC).

(a) Effect of a131 on murine GICs sphere growth. Representative images of DsRed-expressing GICs incubated for 7 days in neural stem cell medium containing DMSO, temozolomide (100 μ M) or a131 (5 μ M).

(b) Effect of a131 on tumor growth in murine brain explants. Schematic description of the experiment (top). Coronal slices established from the brains of tumor-bearing C57BL/6 mice before (Day 0) and after (Day 4) treatment with DMSO or a131 (20 μ M). Red: DsRed-expressing GICs with scale bar: 300 μ m (middle). SVZ: subventricular zone. Tumor growth ratio (Day 4/Day 0) was quantified and mean values with $\pm$ S.D. are shown from triplicated experiments (bottom). Two-tailed unpaired t test was performed to determine the statistical significance.

(c) Effect of a131 on tumor viability in murine brain explants. Immunostaining of cleaved-caspase-3 in coronal brain slices treated with DMSO or a131 (20 μ M) for 4 days. Scale bar: 20 μ m.



Supplementary Figure 4 | Dual inhibitory properties of a131 responsible for its cancer-selective killing efficacy.

(a-c) Normal and transformed BJ cells were treated with a131 and its derivatives at 5 μ M for 48 h.

(a) Immunoblot analysis to determine the ability of 131 and its derivatives to induce mitotic arrest in transformed BJ cells using phospho-histone H3(Ser10) [pH3(Ser10)] and β -actin (loading control) antibodies. The fold induction of band intensities [pH3(Ser10)/ β -actin] as compared to DMSO is plotted with mean values and $\pm$ S.D. (n=3).

(b) BrdU incorporation assay using normal BJ cells as in Fig. 1c. The percentage of cells with BrdU positive population in comparison with DMSO control is shown with mean values and $\pm$ S.D. (n=3).

(c) FACS analysis to determine the percentage of cells in subG1 (<2N). Mean values and $\pm$ S.D. (n=3) are shown. Indicated cells were treated with a131 and its derivatives at 5 μ M (left) or at the indicated concentration (right). Note that only Group 1 compounds retain the ability to selectively kill transformed BJ cells, while compounds in Group 3 killed both normal and transformed cell lines with much less selectivity than those in Group 1.

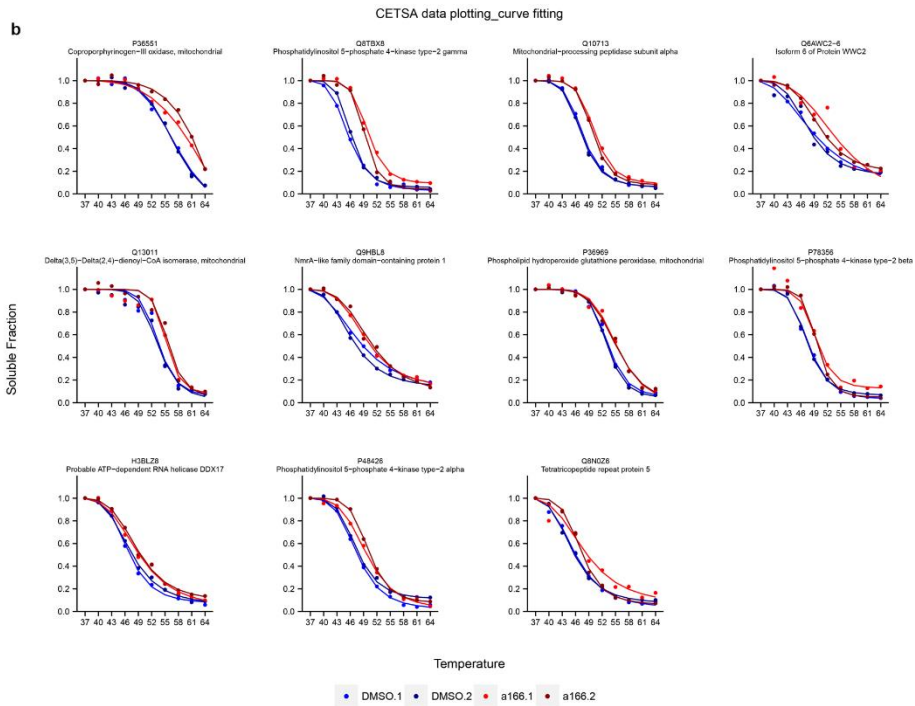
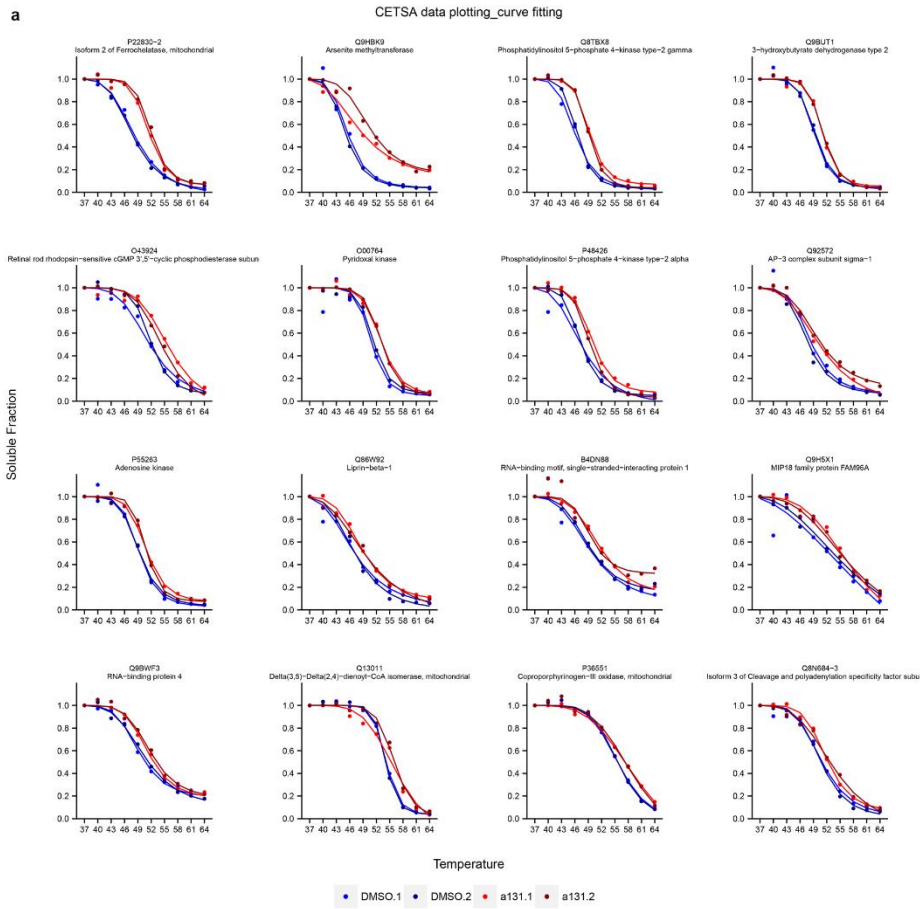
(d) Normal and transformed BJ cells were treated with a159 at a range of different concentrations for 72 h in triplicate and cell viability was determined by MTT assay. Mean values with $\pm$ standard deviation (S.D.) are shown (n=3).

(e-g) Normal and transformed BJ cells were treated with a166 at 5 μ M. 48 h after treatment, cells were further treated with a159 (e), paclitaxel (PTX) (f) or etoposide (g) for additional 48-72 h.

(e, f) FACS analysis of cells stained with Annexin V together with PI. The percentage of double positive cells for Annexin V and PI is shown with mean values and $\pm$ S.D. (n=3).

(g) MTT assay for cell viability. The results are plotted in comparison with DMSO control with mean values and $\pm$ S.D. (n=4).

Where indicated, two-tailed unpaired t test was performed to determine the statistical significance.

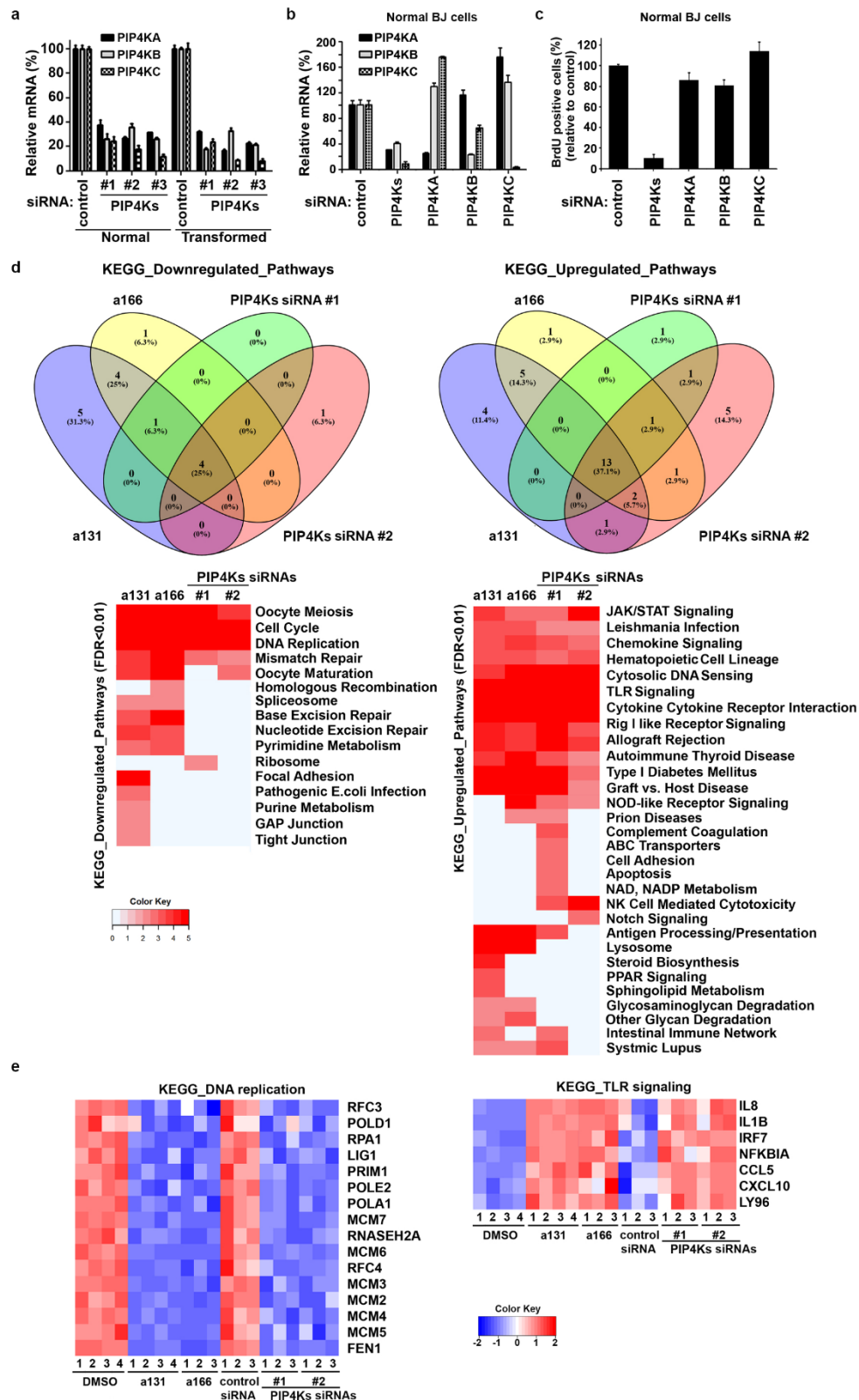


Supplementary Figure 5 | CETSA melt curves for prominent hits with a131 and a166 in normal BJ cell lysates.

(a) Normal BJ cell lysates were treated with vehicle control (DMSO) or a131 compound and then subjected to CETSA treatment. CETSA melt curves for the 16 protein hits that passed the selection criteria are shown. Curves marked in blue represent the DMSO control treated samples and in red show the a131 treated cell lysates.

(b) Normal BJ cell lysates were treated with vehicle control (DMSO) or a166 compound and then subjected to CETSA treatment. CETSA melt curves for the 11 protein hits that passed the selection criteria are shown. Curves marked in blue represent the DMSO control treated samples and in red show the a166 treated cell lysates.

Data is presented as two individual replicates for each condition from one representative experiment.



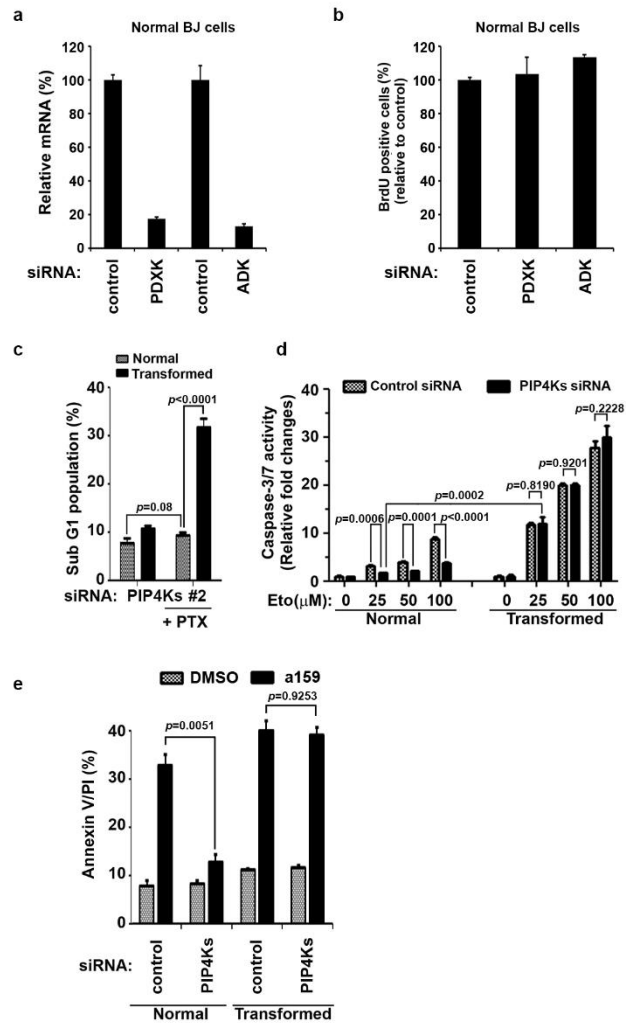
Supplementary Figure 6 | Gene expression similarity between a131 and a166 treatment and PIP4Ks knockdown with phenocopy of growth arrest in normal BJ cells.

(a, b) Quantitative real-time PCR (qRT-PCR) analysis to measure mRNA abundance of individual PIP4K family member in triplicated experiments. Normal and transformed BJ cells were transfected with three different sets of siRNAs to target all PIP4Ks **(a)** or indicated individual siRNA to target individual PIP4K isoform **(b)** as described in the materials and methods.

(c) BrdU incorporation assay as in Fig. 1c. Normal BJ cells were transfected with either control non-silencing or indicated individual or combined PIP4Ks siRNAs for 48h. The percentage of cells with BrdU positive population in comparison with control non-silencing siRNA is shown with mean values and $\pm$ S.D. (n=3).

(d) Gene expression data from normal BJ cells treated with a131 and a166 for 24h or transfected with two different sets of PIP4Ks for 48h were used to identify enriched KEGG pathways. Unique and overlapping pathways were identified through separate 4-way Venn diagrams (top) and heatmaps (bottom) of up- and down-regulated pathway lists, respectively. Pathways that were significant at $FDR < 1\%$ were selected from each of the 4 studies and compared via the Venny software package (<http://bioinforgp.cnb.csic.es/tools/venny/index.html>).

(e) There are a total of 4 down-regulated and 12 up-regulated KEGG pathways at $FDR < 0.01$ across all 4 experiments. In addition to cell cycle pathway as in Fig. 2F, genes contributing to core enrichment of the selected 2 pathways (DNA replication, Toll-like receptor signaling) are shown as examples of the effect for down-regulated and up-regulated pathways. The per-sample expression profiles of these genes are depicted in the heatmap using an intensity-based, row-normalized color scale from blue to red, with blue indicating lower expression.



Supplementary Figure 7 | PIP4Ks knockdown with phenocopy of the chemoprotective effects of a166.

(a) qRT-PCR analysis to measure mRNA abundance of PDXK and ADK in triplicated experiments. Normal BJ cells were transfected with indicated siRNAs to target PDXK and ADK.

(b) BrdU incorporation assay as in Fig. 1c. Normal BJ cells were transfected with either control non-silencing or indicated siRNAs for 48h. The percentage of cells with BrdU positive population in comparison with control non-silencing siRNA is shown with mean values and $\pm$ S.D. (n=3).

(c-e) Normal and transformed BJ cells were transfected with either control non-silencing or PIP4Ks siRNAs for 48h and subsequently treated with paclitaxel (PTX) (d), etoposide (e) or a159 for additional 48-72 h.

(c) FACS analysis with PI staining. The percentage of cells in subG1 ($<2N$) is shown with mean values and $\pm$ S.D. (n=3).

(d) Caspase-3/7 activity assay for apoptosis. The fold induction in comparison with DMSO control is plotted with mean values and $\pm$ S.D. (n=4).

(e) FACS analysis of cells stained with Annexin V together with PI. The percentage of double positive cells for Annexin V and PI is shown with mean values and $\pm$ S.D. (n=3).

Where indicated, two-tailed unpaired t test was performed to determine the statistical significance.

Supplementary Figure 8 | *PIK3IP1* mRNA expression in various normal and Ras- or Raf-mutant cancer cell lines and across indicated cancer-normal and cancer-cancer using TCGA dataset.

(a, b, e) qRT-PCR analysis of *PIK3IP1* mRNA expression in various normal and Ras- or Raf-mutant cancer cell lines.

(a) Endogenous *PIK3IP1* mRNA expression levels.

(b) Indicated cells were treated with DMSO control or a131 at 2.5 and 5 μ M for 24 h.

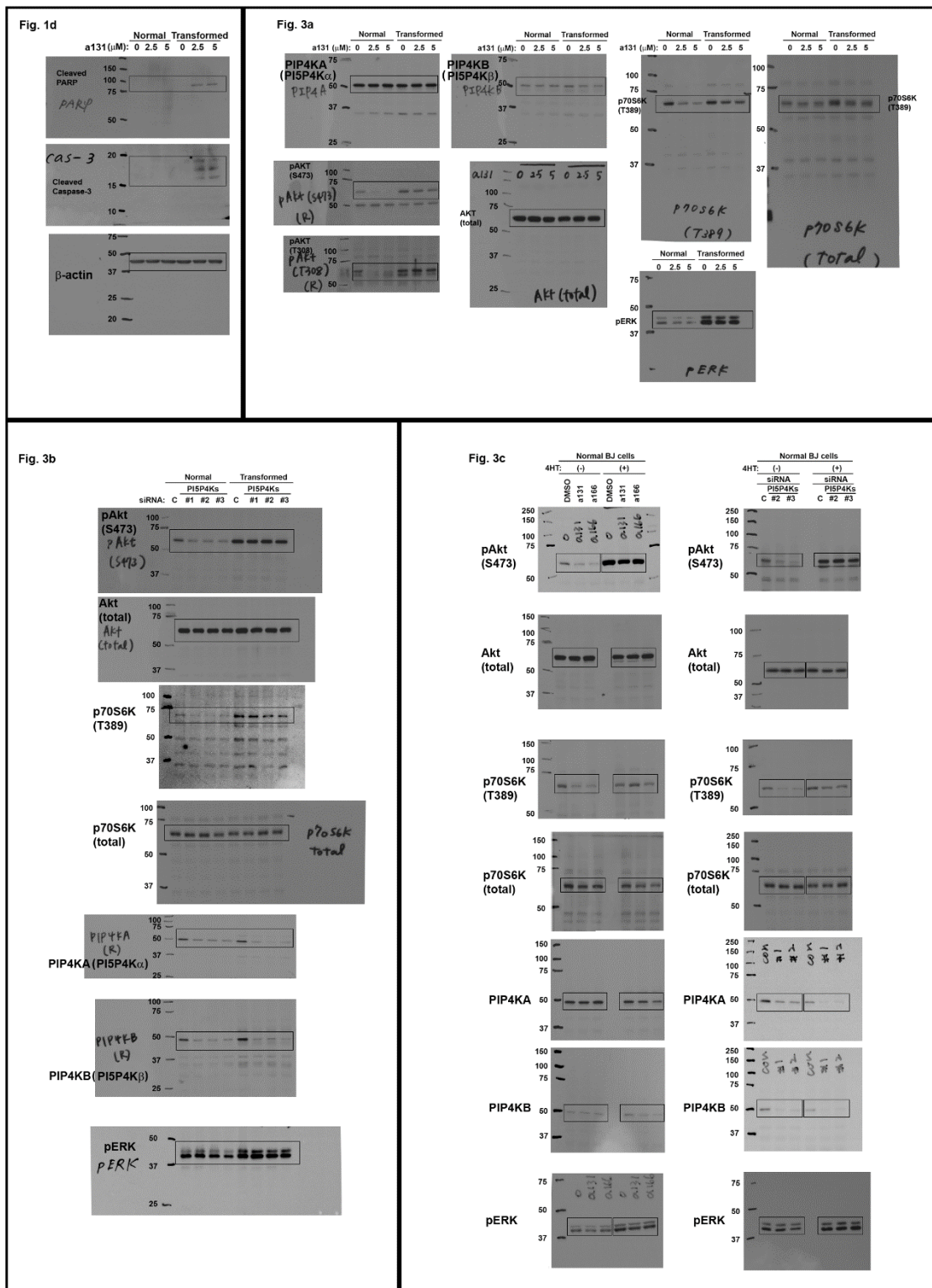
(c, d) Oncomine analysis of *PIK3IP1* gene expression.

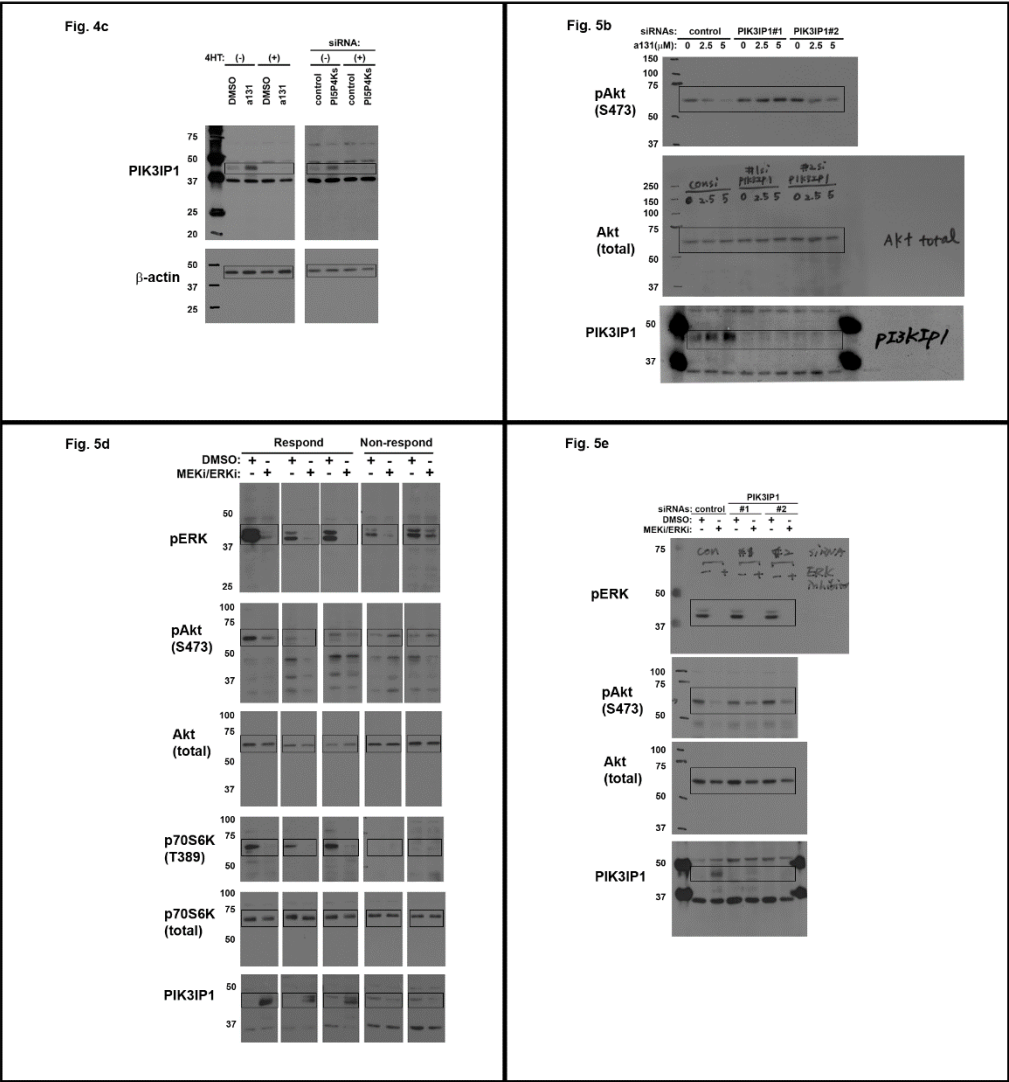
(c) *PIK3IP1* mRNA expression is suppressed in human colorectal and lung adenocarcinomas where Ras mutations and activation of Ras signaling pathways are common compared with their corresponding normal tissues or squamous cell lung carcinoma where Ras mutations are uncommon. Expression microarray results of the TCGA consortium data set were analyzed, and statistical significance was calculated using the Oncomine website (www.oncomine.org).

(d) Negative correlations between *PIK3IP1* mRNA expression and Ras mutation status in human colorectal and lung adenocarcinomas.

Box plots show differences in mRNA expression across indicated cancer-normal and cancer-cancer. Data are presented as box plot distribution (line = median value). Numbers represent samples analyzed.

(e) Pharmacological inhibition of MEK/ERK attenuates Ras- and Raf-mediated suppression of *PIK3IP1*. Various Ras- or Raf-mutant cancer cell lines were treated with MEK inhibitor (MEKi) U0126 and/or ERK inhibitor (ERKi) SCH772984 for 24h and *PIK3IP1* mRNA expression was measured by qRT-PCR. Of note, increase in *PIK3IP1* mRNA expression was much prominent in Raf-mutant cancer cells, suggesting the high MAPK activity is responsible for the suppression of *PIK3IP1*.





Supplementary Figure 9 | Uncut blots. The black box regions are presented in the indicated figures in the manuscript.