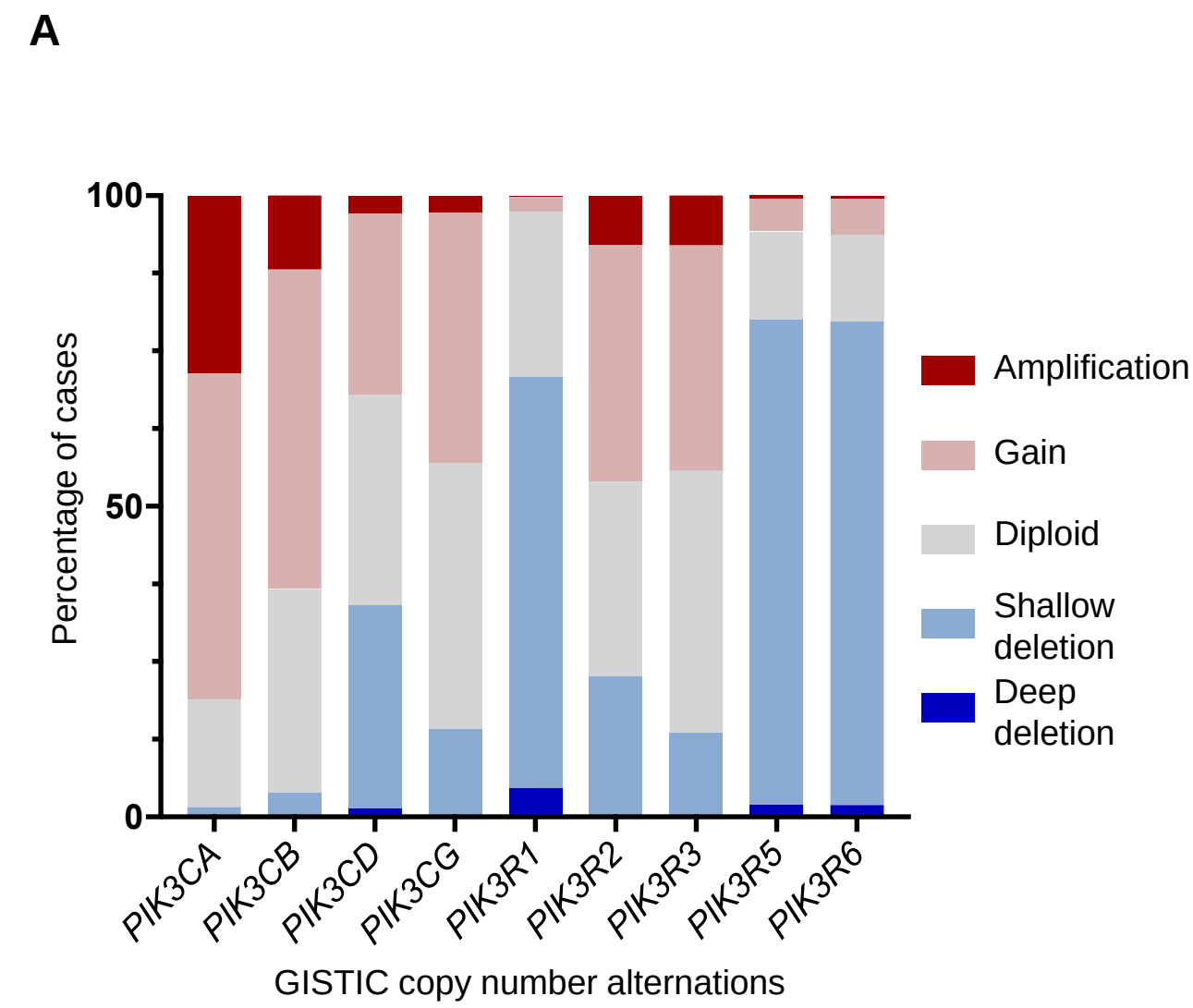
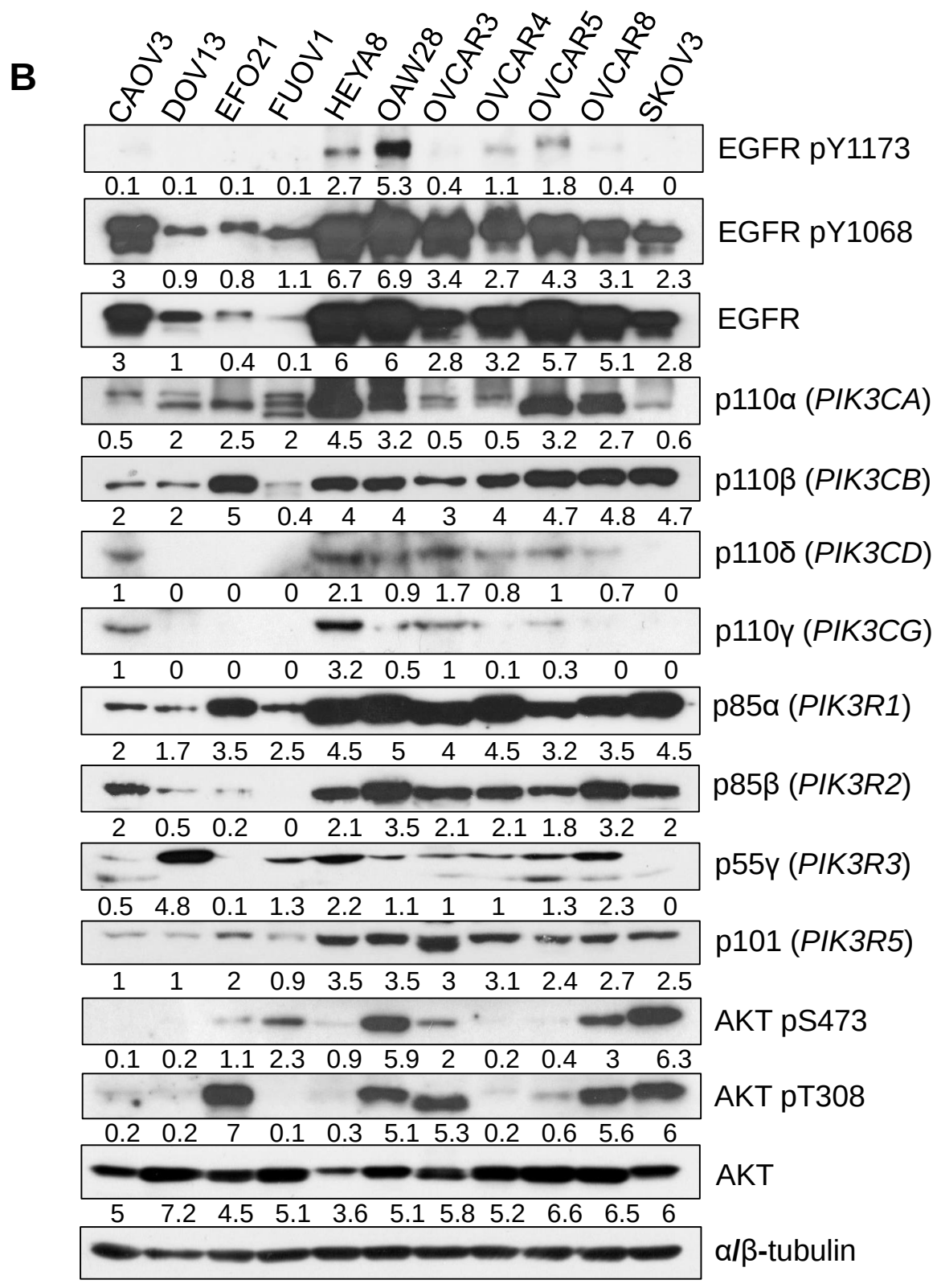
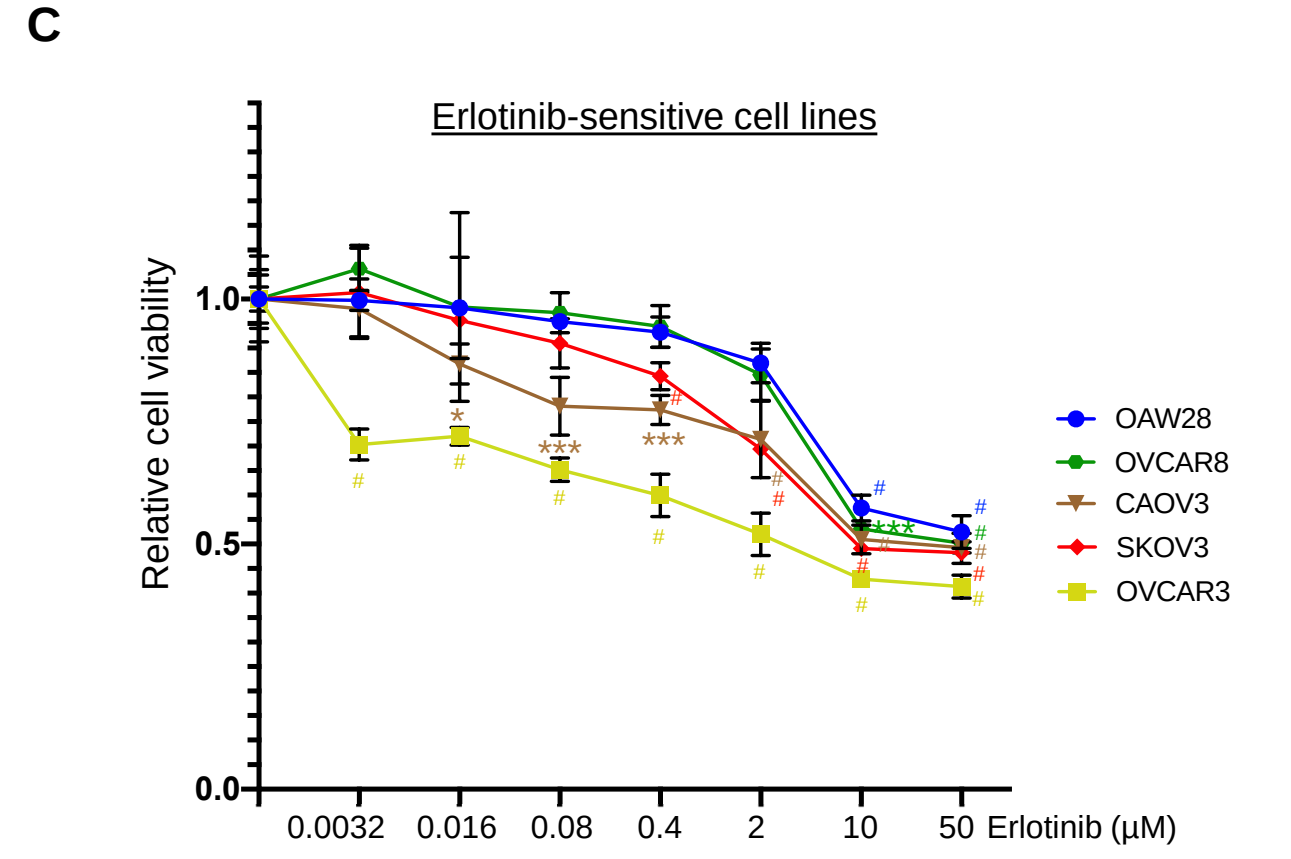
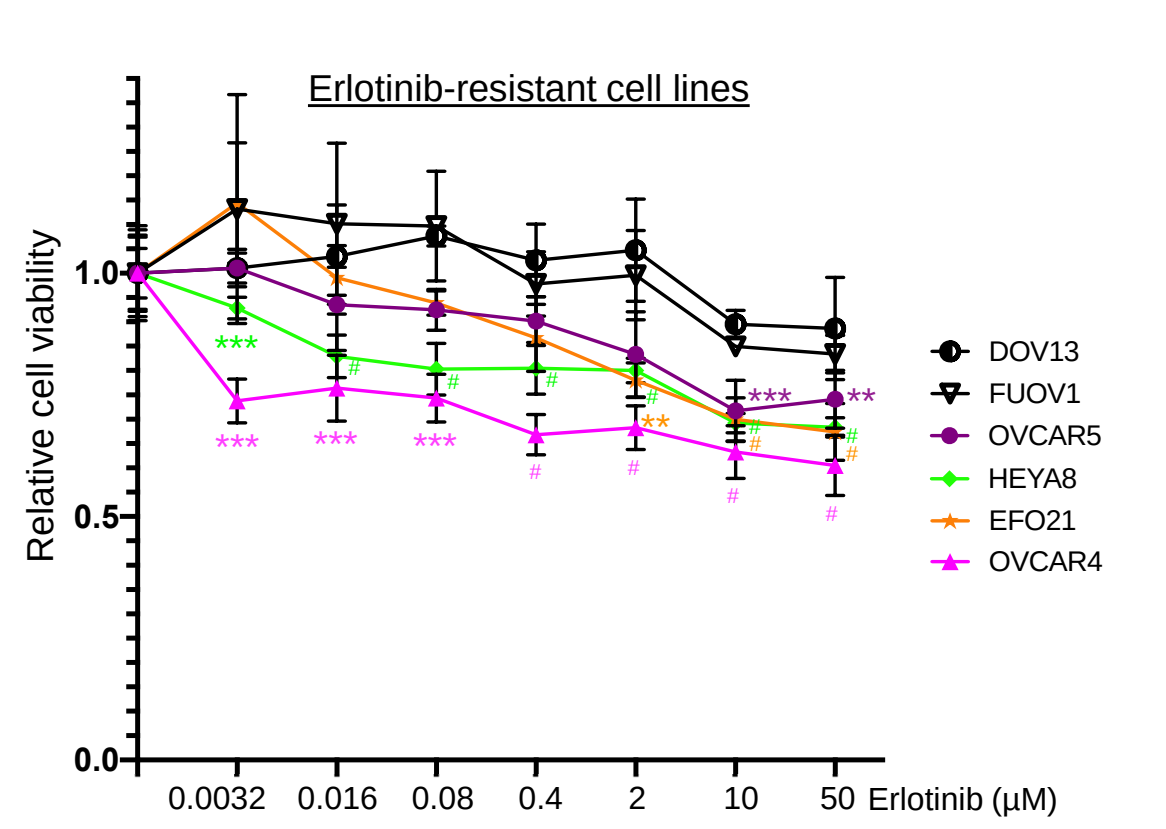
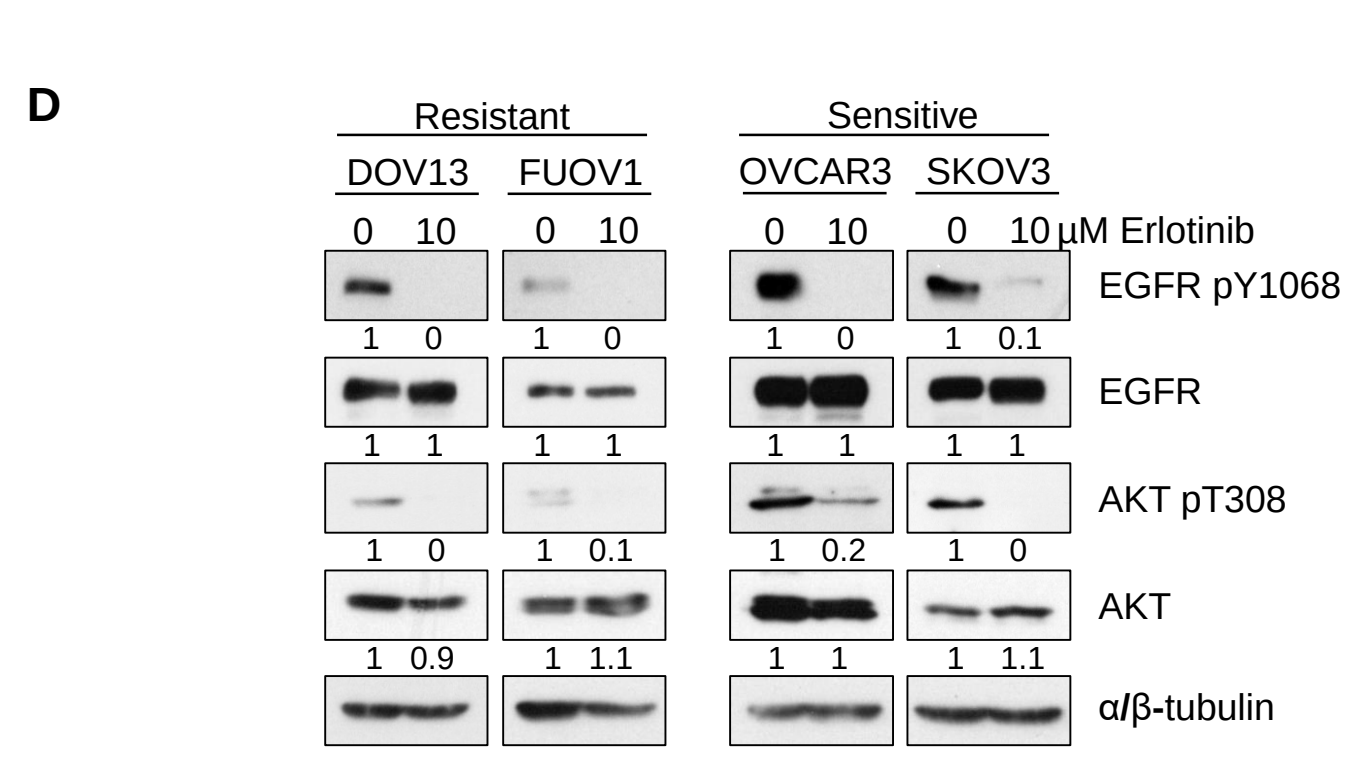
Supplementary Figure 1







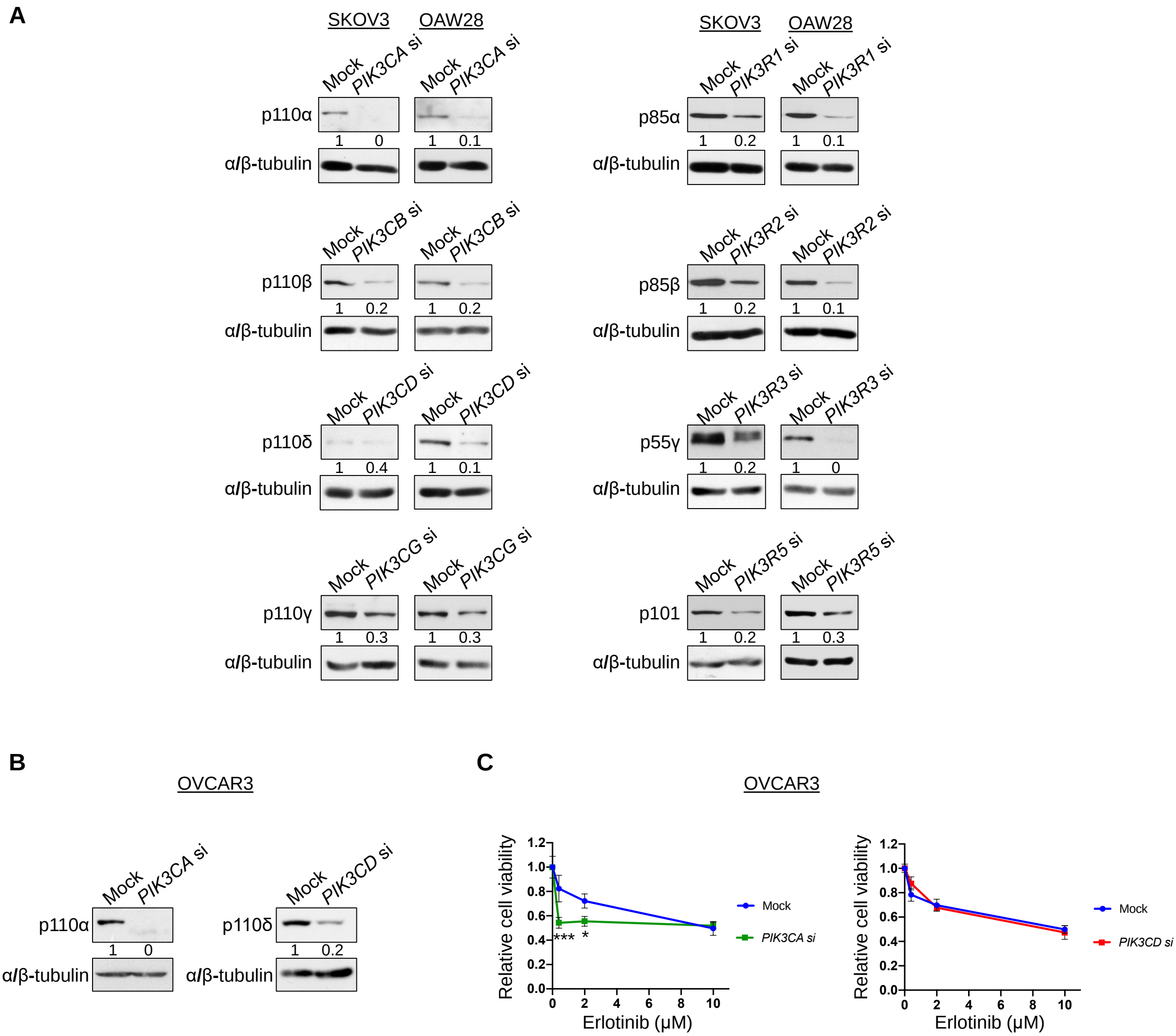




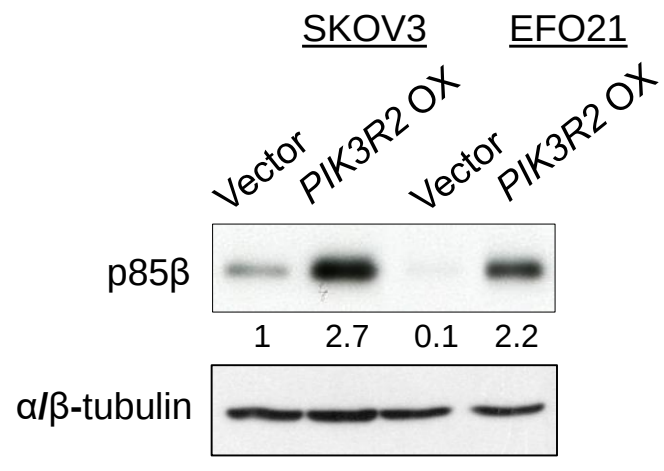
Supplementary Figure 1. Expression levels of EGFR and PI3K isoforms as well as responses to erlotinib of ovarian cancer cell lines.

(A) The distribution of copy number alterations (from GISTIC analysis) of the indicated genes across TCGA serous ovarian cancer cases (n = 311). (**B)** Total protein lysates were harvested from 11 serous ovarian cancer cells. Protein levels were assessed using the indicated antibodies by Western blotting. α/β-tubulin was a loading control. (**C)** Ovarian cancer cells were treated with a serial dilution of erlotinib for 72 hr prior to cell viability assay. Data are shown as mean ± SD of three independent experiments in triplicate. *Left*, cell lines that showed ≥50% viability inhibition after 50 μM erlotinib treatment were defined as "sensitive". *Right*, cell lines whose viability inhibition did not reach 50% were "resistant". *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$; #, $p<0.0001$ compared with DMSO control using ordinary one-way ANOVA with Tukey's multiple comparison test. (**D)** Two erlotinib-resistant cell lines (DOV13 and FUOV1) and two erlotinib-sensitive cell lines (SKOV3 and OVCAR3) were treated with 10 μM erlotinib. Western blotting was performed to confirm inhibition efficiency. Representative blots of three independent experiments are shown. The numbers below the blots indicate the mean densitometry values normalized to those of α/β-tubulin of the three experiments.

Supplementary Figure 2

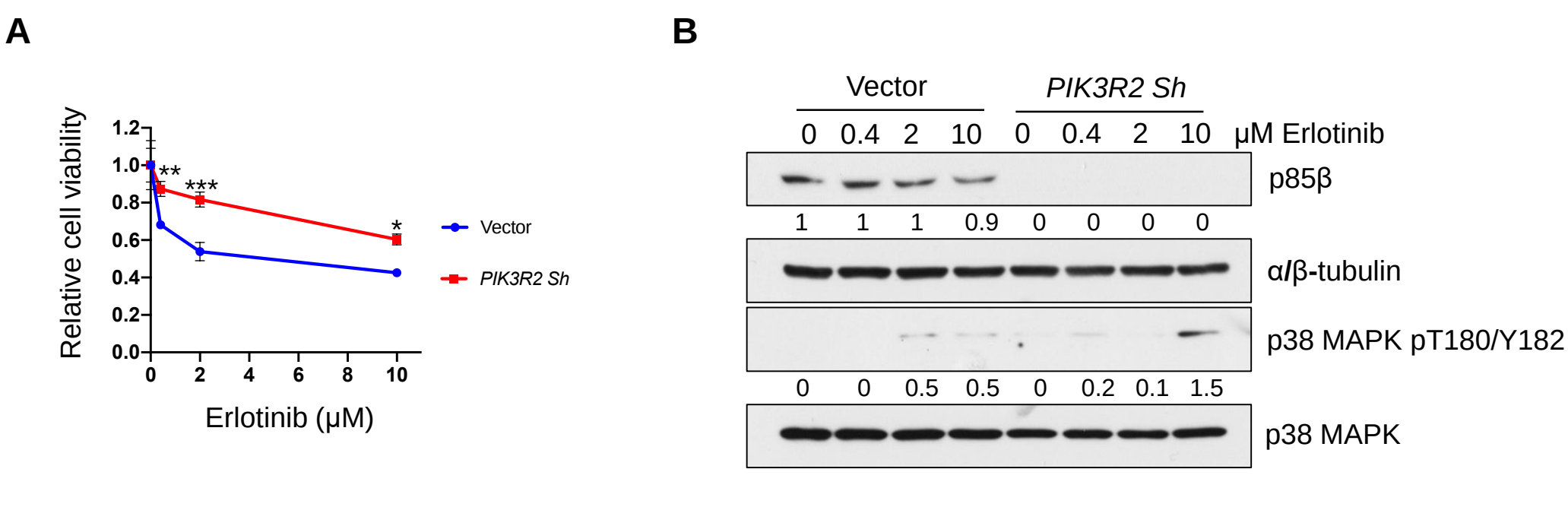


Supplementary Figure 2. Verification of the efficiency of siRNAs targeting the PI3K isoforms and the effects of siRNA on erlotinib responses of ovarian cancer cell lines.
(A) SKOV3 and OAW28 cells or (**B**) OVCAR3 cells were transfected with siRNA for 72 hr and Western blotting was performed to evaluate knockdown efficiency. Representative blots of three independent experiments are shown. The numbers below the blots indicate the mean densitometry values normalized to those of α/β-tubulin of the three experiments. (**C)** OVCAR3 cells transfected with siRNA for 72 hr were treated with erlotinib for another 72 hr prior to viability assays. Data are shown as mean ± SD of three independent experiments in triplicate. *, $p<0.05$; ***, $p<0.001$ by two-way ANOVA with Sidak's multiple comparison test.



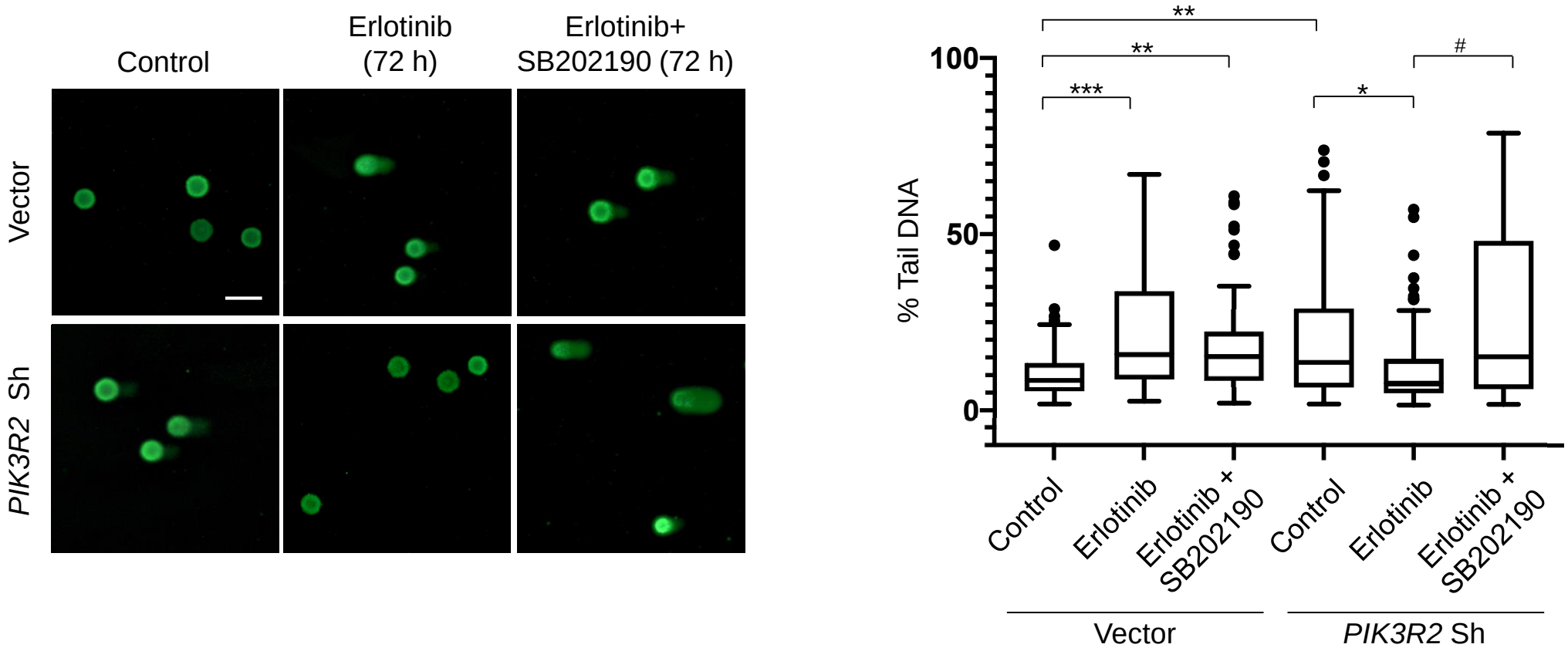
Supplementary Figure 3. Verification of *PIK3R2* overexpression. *PIK3R2* expression plasmid (OX) or empty vector was introduced into SKOV3 or EFO21 cells. After 72 hr, Western blotting was performed to evaluate overexpression efficiency.

Supplementary Figure 4



Supplementary Figure 4. Cells with shRNA-mediated *PIK3R2* knockdown, which have reduced response to erlotinib, show p38 MAPK activation upon erlotinib treatment. (A-B) SKOV3 cells stably expressing vector control or *PIK3R2* shRNA (sh) upon increasing doses of erlotinib treatment for 72 hr were subjected to (A) in vitro cell viability assay or (B) Western blotting. Cell viability data are shown as mean ± SD of three independent experiments in triplicate. *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$ by two-way ANOVA with Sidak's multiple comparison test. Representative blots of three independent experiments are shown. The numbers below the blots indicate the mean densitometry values normalized to those of α/β-tubulin or total p38 MAPK of the three experiments.

Supplementary Figure 5



Supplementary Figure 5. Erlotinib reduces the amount of unrepaired DNA in *PIK3R2*-depleted cells. This reduction is reversed by p38 MAPK inhibitor SB202190.

SKOV3 cells stably expressing *PIK3R2* shRNA or vector control were treated with erlotinib alone (10 μM) or in combination with SB202190 (10 μM) for 72 hr. DNA damage was visualized by comet assay (*left*). Scale bar, 100 μm. The amount of damaged DNA was expressed as percentage of DNA in comet tail (% tail DNA) (*right*). The box and whisker plot was plotted by Graphpad Prism software using the Tukey method. *, $p<0.05$; ***, $p<0.001$; #, $p<0.0001$ using ordinary one-way ANOVA for comparison within groups (mock or *PIK3R2* shRNA) and two-way ANOVA for comparison between groups (mock vs *PIK3R2* shRNA) with Sidak's multiple comparison test.

Supplementary Table 1. Sequences of siRNA and shRNA

	Gene Symbol	Target Protein	Sequence	Catalog number
siRNA	*PIK3CA*	p110α	GUGGUAAAGUUCCCAGAUA	J-003018-13
	PIK3CB	p110β	GGAUUCAGUUGGAGUGAUU	J-003019-10
	PIK3CD	p110δ	GCGUGGGCAUCAUCUUUAA	J-006775-11
	PIK3CG	p110γ	CCCGAAAGCUUUAGAGUUC	J-005274-08
	PIK3R1	p85α	CCAACAACGGUAUGAAUAA	J-003020-15
	PIK3R2	p85β	GCGCCCAGCUUAAGGUCUA	J-003021-09
	PIK3R3	p55γ	GGAUAUCAAUCGAGUACAA	J-019546-05
	PIK3R5	p101	CGACAGAGAUCUUCAUCCA	J-020619-08
shRNA	*PIK3R2*	p85β	CAGATGAAGCGTACTGCAATT	

Supplementary Table 2. Antibodies used in this study

Antibodies	Abbreviation	Source	Catalog number	Dilution	
				Western blot	Immunofluorescence
Anti-phospho-EGFR (Tyr1068)	EGFR pY1068	Cell Signaling	2234	1:1000	
Anti-EGFR	EGFR	Epitomics	1902-1	1:4000	
Anti-p110γ	p110γ	Cell Signaling	5405	1:1000	
Anti-p110δ	p110δ	Abcam	ab1678	1:2200	
Anti-p110β	p110β	Santa Cruz	sc-376412	1:100	
Anti-p110α	p110α	Cell Signaling	4255	1:750	
Anti-p101	p101	EMD Millipore	07-281	1:3000	
Anti-p85β	p85β	Santa Cruz	sc-56934	1:200	
Anti-p85α	p85α	Santa Cruz	sc-71892	1:200	
Anti-p55γ	p55γ	Santa Cruz	sc-376615	1:750	
Anti-phosopho-AKT (Ser473)	AKT pS473	Cell Signaling	9271	1:1000	
Anti-phosopho-AKT (Thr308)	AKT pT308	Santa Cruz	sc-271966	1:1000	
Anti-AKT	AKT	Cell Signaling	4691	1:3000	
Anti-α/β-tubulin	α/β-tubulin	Cell Signaling	2148	1:5000	
Anti-MEK1/2 (Ser217/Ser221)	MEK1/2 pS217/221	Cell Signaling	9154	1:1000	
Anti-MEK1	MEK1	Santa Cruz	6250	1:250	
Anti-phospho-p38 (Thr180/Tyr182)	p38 MAPK pT180/Y182	cell Signaling	9211	1:1000	
Anti-p38 MAPK	p38 MAPK	Cell Signaling	9212		1:40
Anti-phospho-SAPK/JNK (Thr183/Tyr185)	JNK pT183/Y185	Cell Signaling	9255	1:1000	
Anti-SAPK/JNK	JNK	Cell Signaling	9252	1:1000	

Anti-phospho-ERK1/2 (Thr202/Tyr204)	ERK1/2 pT202/Y204	Cell Signaling	9101	1:2500	
Anti- ERK1/2	ERK1/2	Cell Signaling	9102	1:5000	
Anti-53BP1	53BP1	Cell Signaling	4937	1:1000	1:75
Anti-RAD51	RAD51	EMD Millipore	PC130	1:1000	1:200
Anti-PARP	PARP	Cell Signaling	9542	1:1000	
Anti-lamin A/C	Lamin A/C	Cell Signaling	2032	1:1000	

Supplementary Table 3. Mutation status of *EGFR* and PI3K isoforms across 11 serous ovarian cancer cell lines

	EGFR	*PIK3CA*	*PIK3CB*	*PIK3CD*	*PIK3CG*	*PIK3R1*	*PIK3R2*	*PIK3R3*	*PIK3R5*	*PIK3R6*
CAOV3	R255Q									
DOV13										
EFO21										
FUOV1										
HEY8A										
OAW28										
OVCAR3						Splice site*				
OVCAR4			K718R							
OVCAR5										
OVCAR8										
SKOV3		H1047R								

Data were extracted from the Cancer Cell Line Encyclopedia (CCLE).

* The *PIK3R1* splice site mutation leads to exon 13 skipping.