

Overcoming gefitinib resistance and metastasis in adenocarcinoma through synergistic PI3K–AKT–ERK blockade: Integrated rational co-delivery nanoplatform and multimodel validation

Supplementary File

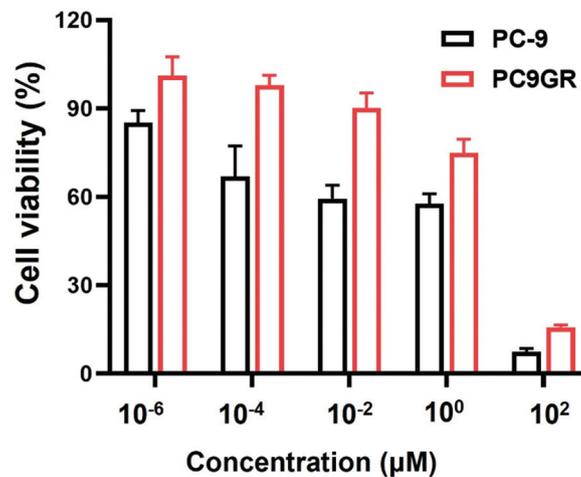


Figure S1. Cell viability of gefitinib-resistant PC-9 (PC9GR) cells and parental PC-9 cells following treatment at different concentrations of gefitinib

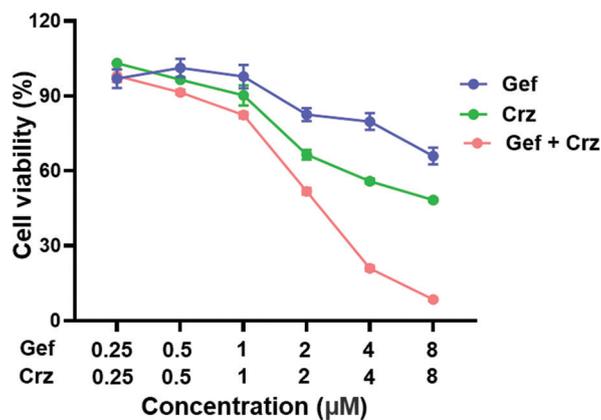


Figure S2. Cell viability of Gef-resistant PC-9 cells following treatment with different formulations at different concentrations. Abbreviations: Crz: Crizotinib; Gef: Gefitinib.

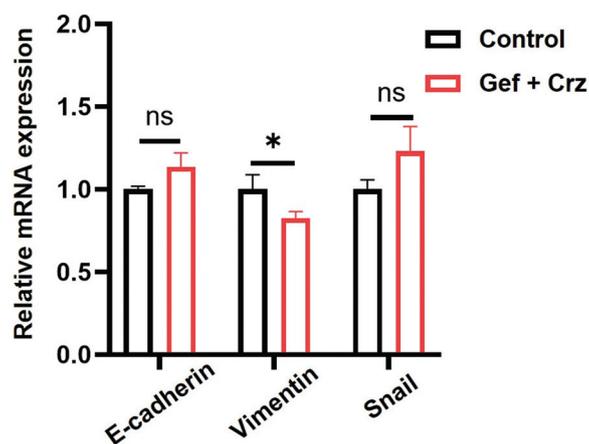


Figure S3. Relative mRNA expression of key EMT markers following Gef + Crz combination treatment
Abbreviations: Crz: Crizotinib; Gef: Gefitinib.

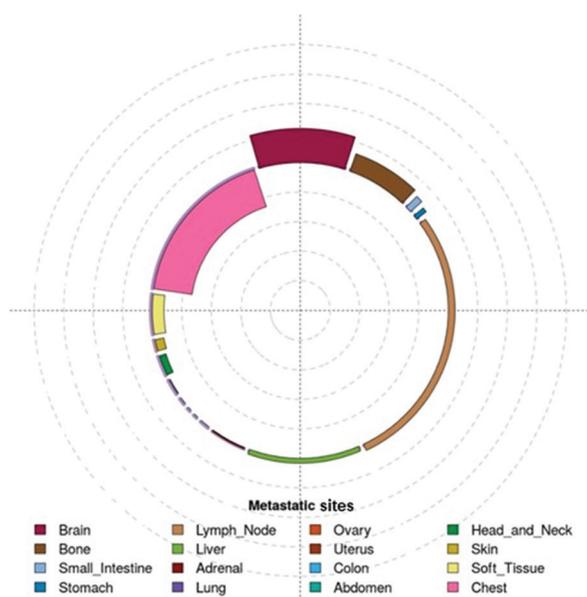


Figure S4. Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)-related metastasis radar plot in lung cancer. The PI3K/AKT signaling pathway is implicated in the development of distal organ metastasis.

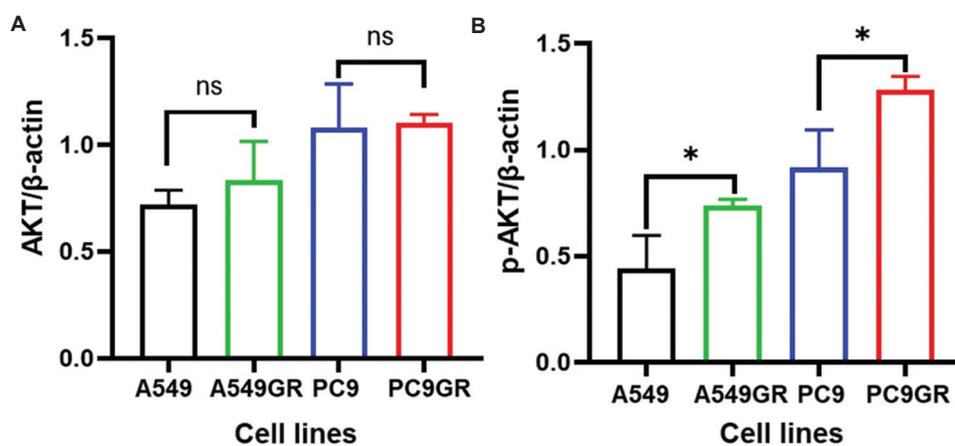


Figure S5. Gefitinib-resistant cells exhibit overactivation of the AKT signaling pathway. Quantitative analysis of (A) AKT/ β -actin and (B) phosphorylated (p)-AKT/ β -actin protein ratios. Data are presented as mean \pm standard deviation. Statistical significance was assessed using an unpaired Student's *t*-test. * $p < 0.05$ indicates statistical significance, whereas "ns" indicates no significant difference.
Abbreviations: A549GR: Gefitinib-resistant A549 cell; AKT: Protein kinase B; PC9GR: Gefitinib-resistant PC-9 cell.

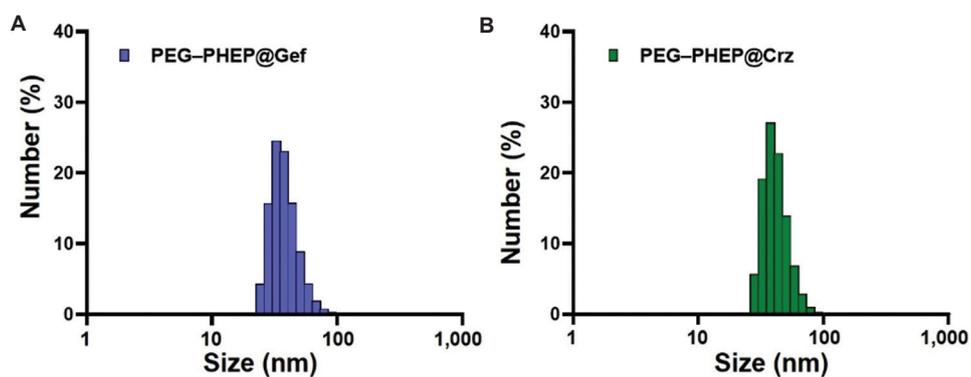


Figure S6. Number-weighted particle size distribution of (A) PEG-PHEP@Gef and (B) PEG-PHEP@Crz
Abbreviations: Crz: Crizotinib; Gef: Gefitinib; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

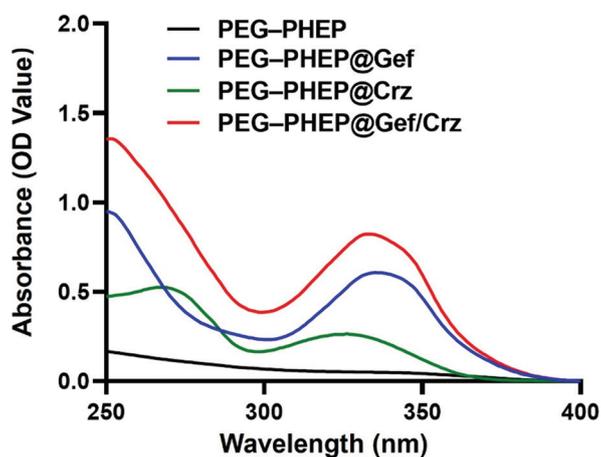


Figure S7. Ultraviolet-visible spectra of PEG-PHEP@Gef, PEG-PHEP@Crz, and PEG-PHEP@Gef/Crz
Abbreviations: Crz: Crizotinib; Gef: Gefitinib; OD: Optical density; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

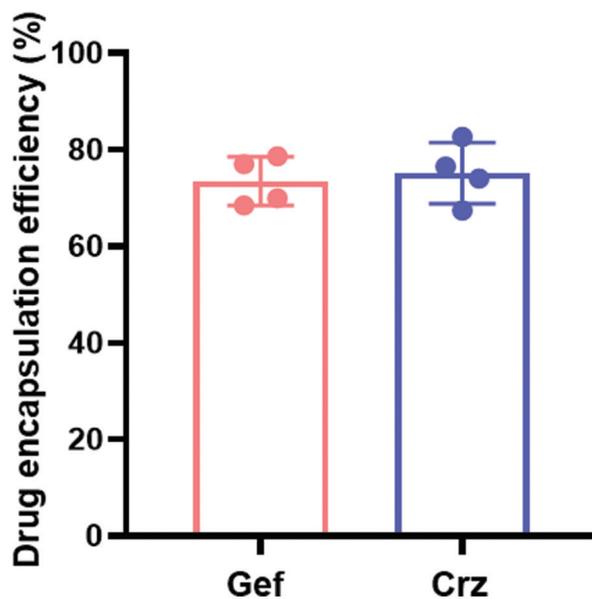


Figure S8. Drug encapsulation efficiency of PEG-PHEP@Gef/Crz
Abbreviations: Crz: Crizotinib; Gef: Gefitinib; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

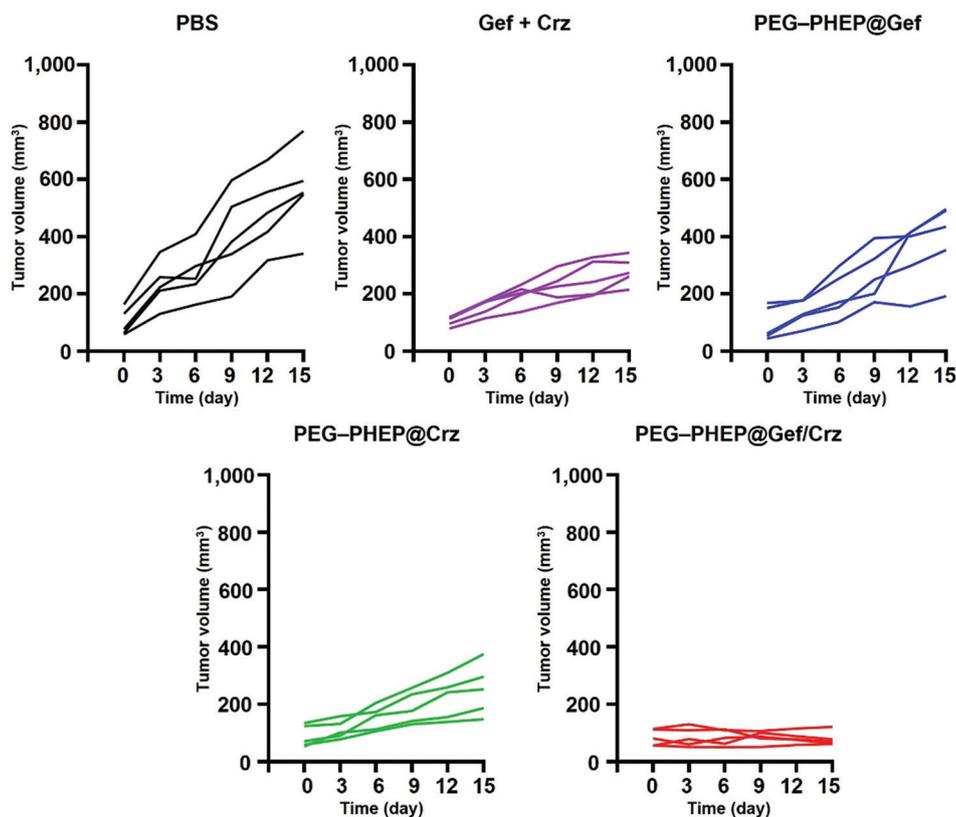


Figure S9. Growth curves of tumors following treatment with different formulations

Abbreviations: Crz: Crizotinib; Gef: Gefitinib; PBS: Phosphate-buffered saline; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

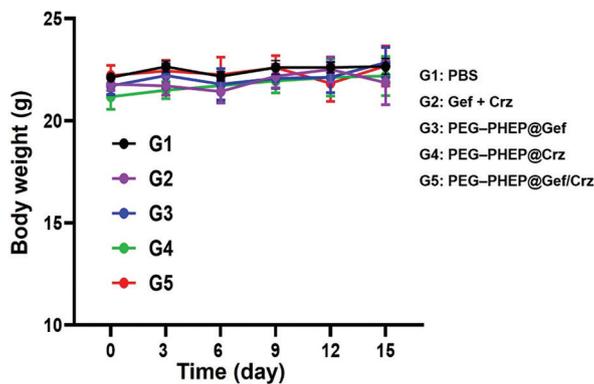


Figure S10. Body weight curves of tumor-bearing mice over 15 days following treatment with different formulations

Abbreviations: Crz: Crizotinib; Gef: Gefitinib; PBS: Phosphate-buffered saline; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

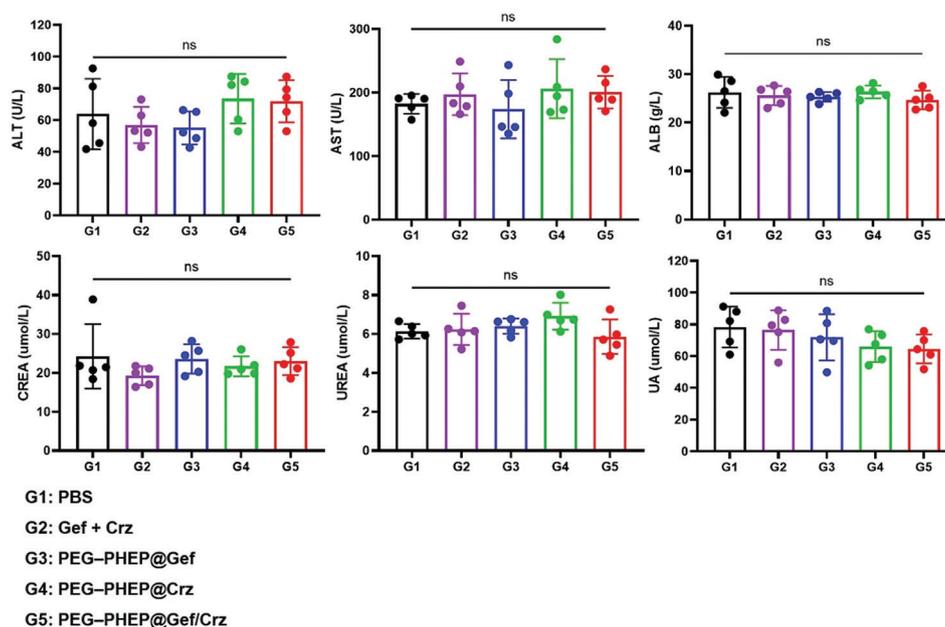
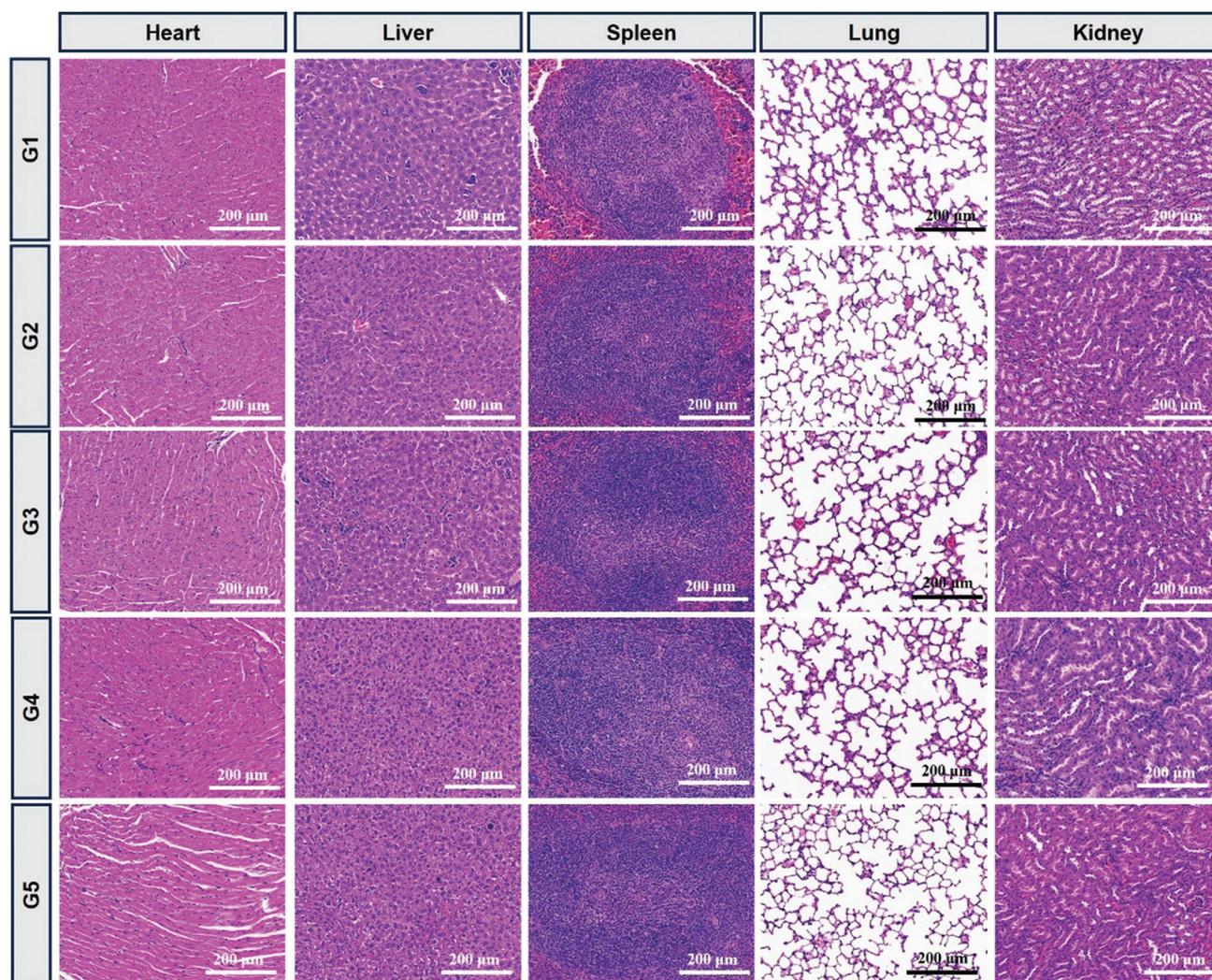


Figure S11. Serum biochemical analysis of tumor-bearing mice following treatment with different formulations

Abbreviations: ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate transaminase; CREA: Creatinine; Crz: Crizotinib; Gef: Gefitinib; PBS: Phosphate-buffered saline; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate); UA: Uric acid; UREA: Urea nitrogen. Data are presented as mean \pm standard deviation. Statistical significance was assessed using an unpaired Student's *t*-test. "ns" indicates no significant difference.

Table S1. Forward and reverse primer sequences for E-cadherin, N-cadherin, Vimentin, Snail, and the reference gene *GAPDH* used for quantitative real-time polymerase chain reaction analysis

Gene	Forward primer (5'→3')	Reverse primer (3'→5')
E-cadherin (<i>CDH1</i>)	AGGCCAAGCAGCAGTACATT	CATTCACATCCAGCACATCC
N-cadherin (<i>CDH2</i>)	TTTGAGGGCACATGCAGTAG	ACTGTCCCATTCCAAACCTG
Vimentin (<i>VIM</i>)	CGAAACTTCTCAGCATCAG	GCAGAAAGGCACTTGAAGC
Snail (<i>SNAI1</i>)	CCTCGCTGCCAATGCTCATCTG	GCTCTGCCACCCTGGGACTC
<i>GAPDH</i>	GGGAAGGTGAAGTCCGAGT	GGGGTCATTGATGGCAACA



G1: PBS

G2: Gef + Crz

G3: PEG-PHEP@Gef

G4: PEG-PHEP@Crz

G5: PEG-PHEP@Gef/Crz

Figure S12. Representative images of hematoxylin and eosin staining of major tissues such as heart, liver, spleen, lung, and kidney tissues following treatment with different formulations (scale bar = 200 μ m; magnification = 40 \times)

Abbreviations: Crz: Crizotinib; Gef: Gefitinib; PBS: Phosphate-buffered saline; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

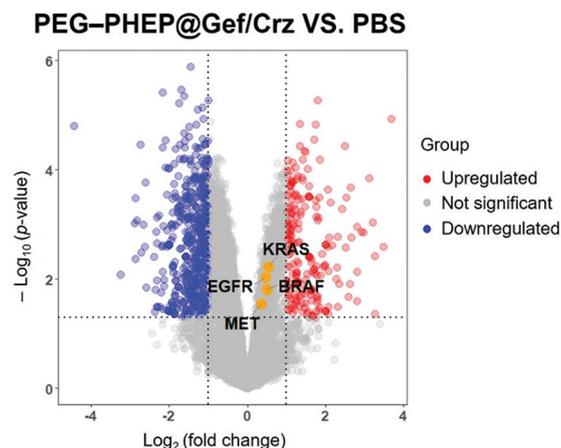


Figure S13. Volcano plot showing differential gene expression between the PEG-PHEP@Gef/Crz and PBS groups. Key genes (*EGFR*, *MET*, *KRAS*, and *BRAF*) are labeled in yellow to indicate their relative expression changes and statistical significance. Abbreviations: Crz: Crizotinib; Gef: Gefitinib; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).

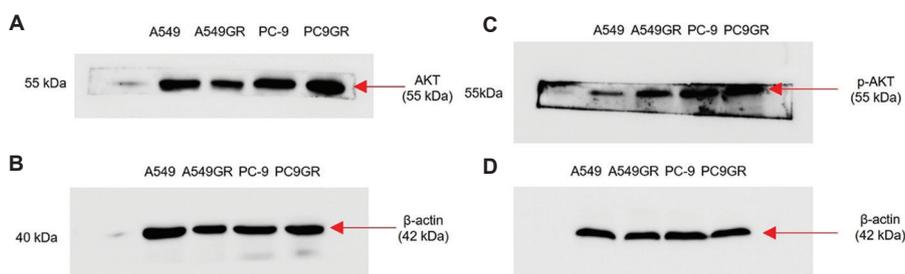


Figure S14. Original Western blot images of Figure 1F for (A) AKT (55 kDa), (B) β -actin (42 kDa), (C) p-AKT (55 kDa), and (D) β -actin (42 kDa) in Figure 1F. Abbreviations: A549GR: Gefitinib-resistant A549 cell; AKT: Protein kinase B; p-AKT: Phosphorylated protein kinase B; PC9GR: Gefitinib-resistant PC-9 cell.

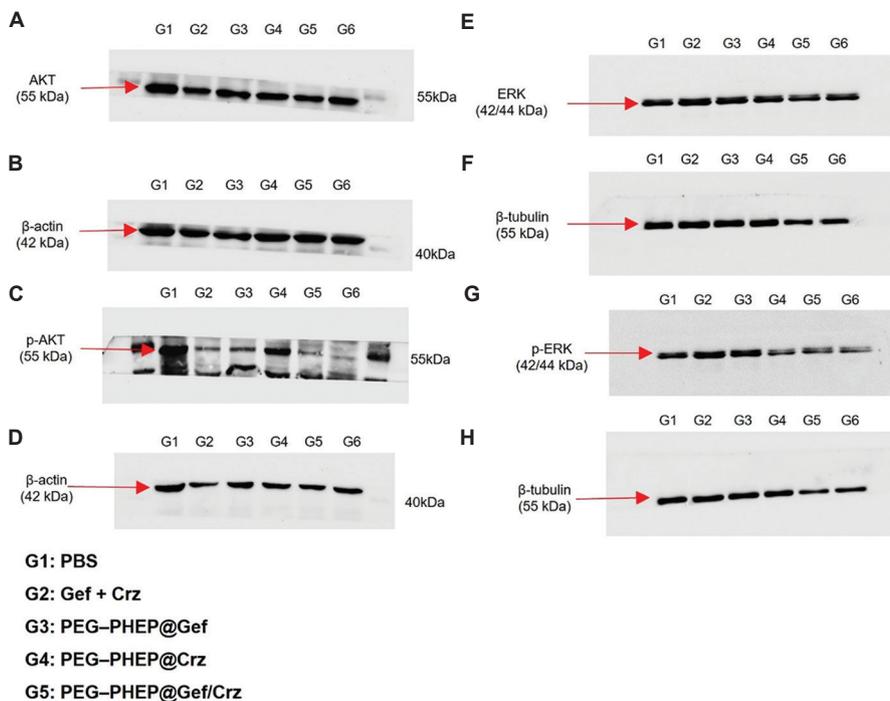


Figure S15. Original Western blot images of Figure 3C for (A) AKT (55 kDa), (B) β -actin (42 kDa), (C) p-AKT (55 kDa), (D) β -actin (42 kDa), (E) ERK (42/44 kDa), (F) β -tubulin (55 kDa), (G) p-ERK (42/44 kDa), and (H) β -tubulin (55 kDa). Abbreviations: AKT: Protein kinase B; Crz: Crizotinib; ERK: Extracellular signal-regulated kinase; Gef: Gefitinib; p-AKT: Phosphorylated protein kinase B; PEG: Poly (ethylene glycol); PHEP: Poly (hexyl ethylene phosphate).