

# **Protein tyrosine kinase 2: A novel therapeutic target to overcome acquired EGFR-TKI resistance in non-small cell lung cancer**

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## **Additional file 1**

**Figure S1** – PC-9 and PC-9/PEM do not express FGFR1 or FGFR4 proteins.

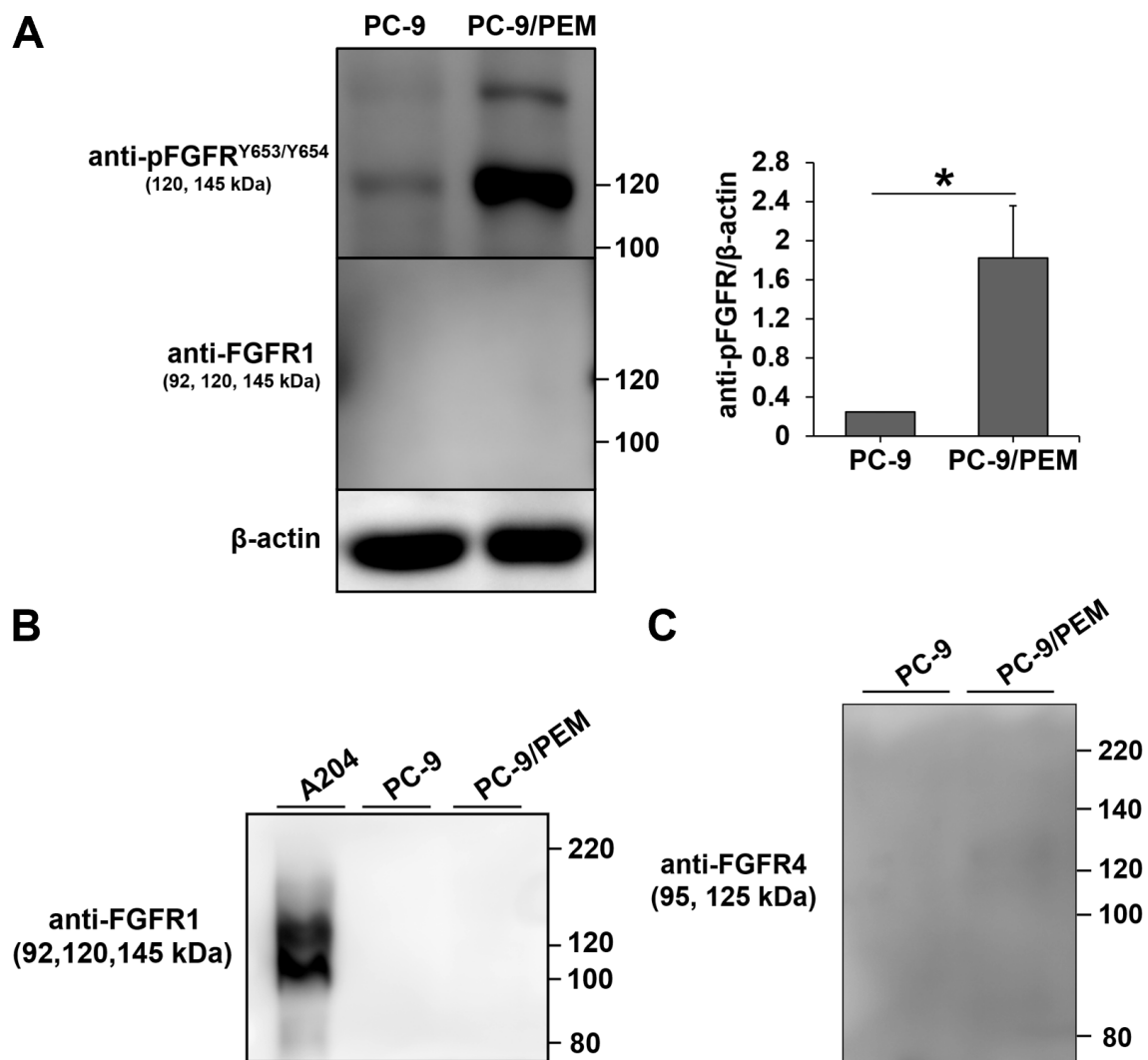
**Figure S2** – PD173074 sensitized PC-9/PEM to erlotinib but BLU-554 and nintedanib did not.

**Figure S3** – Representative pictures and body weights of xenografts.

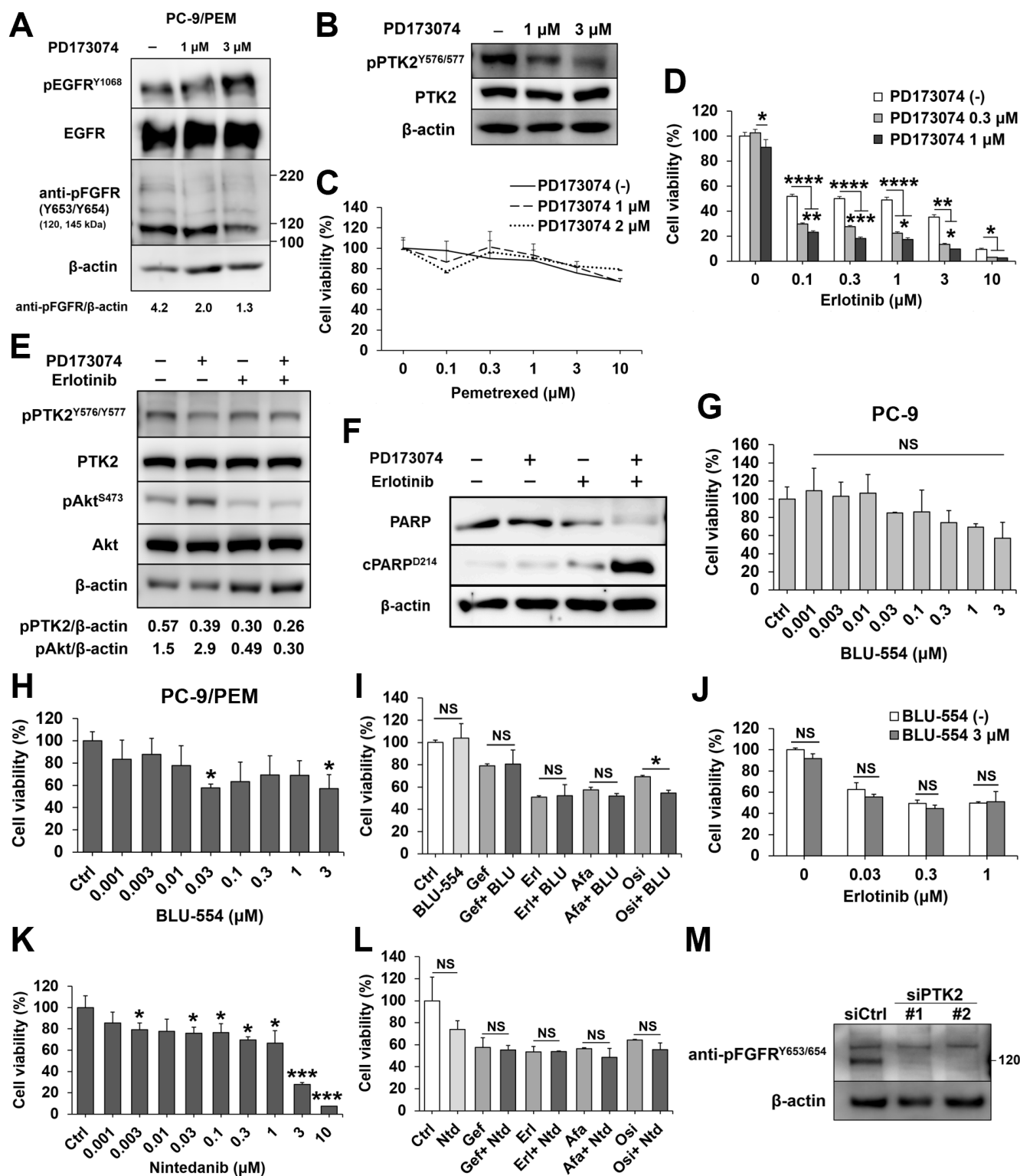
**Figure S4** – Establishment of erlotinib-resistant NSCLC cell lines.

**Figure S5** – Sequence alignment between PTK2 (Y407 to F729) and FGFR1 (Y463 to L819).

**Table S1** – The sequences for primers used in RT- qPCR.

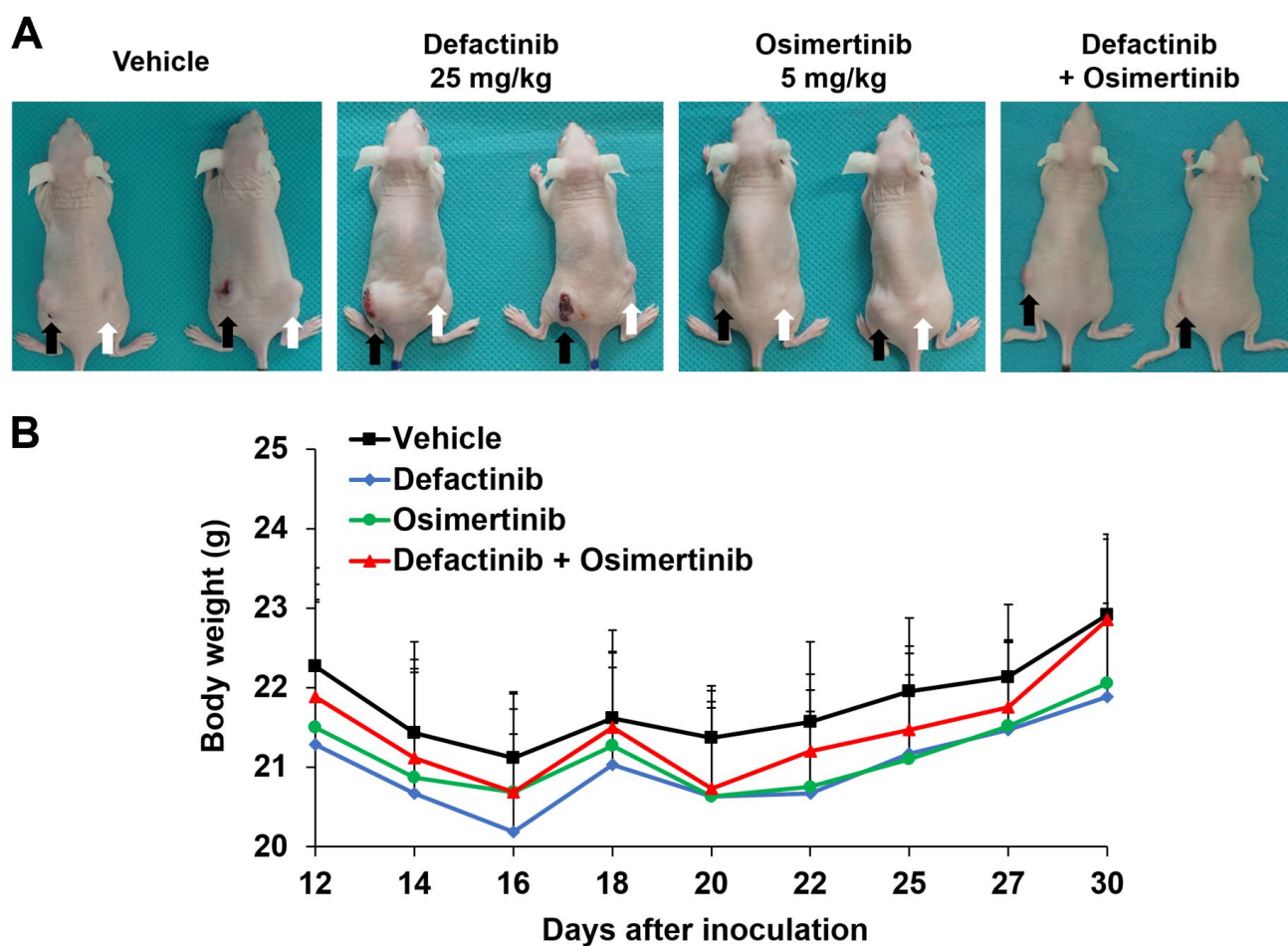


**Figure S1** PC-9 and PC-9/PEM do not express FGFR1 or FGFR4 proteins. **A**, Representative immunoblots of protein binding to anti-pFGFR<sup>Y653/Y654</sup> and total FGFR1 in PC-9 and PC-9/PEM cells and ratio of phosphorylated protein to β-actin. Data are means (SD), n = 3. \*, *P* < 0.05 by Student's *t*-test. **B**, Immunoblots of total FGFR1 in PC-9 and PC-9/PEM cells. A204 protein lysate was used as the positive control. **C**, immunoblots of total FGFR4 in PC-9 and PC-9/PEM cells.

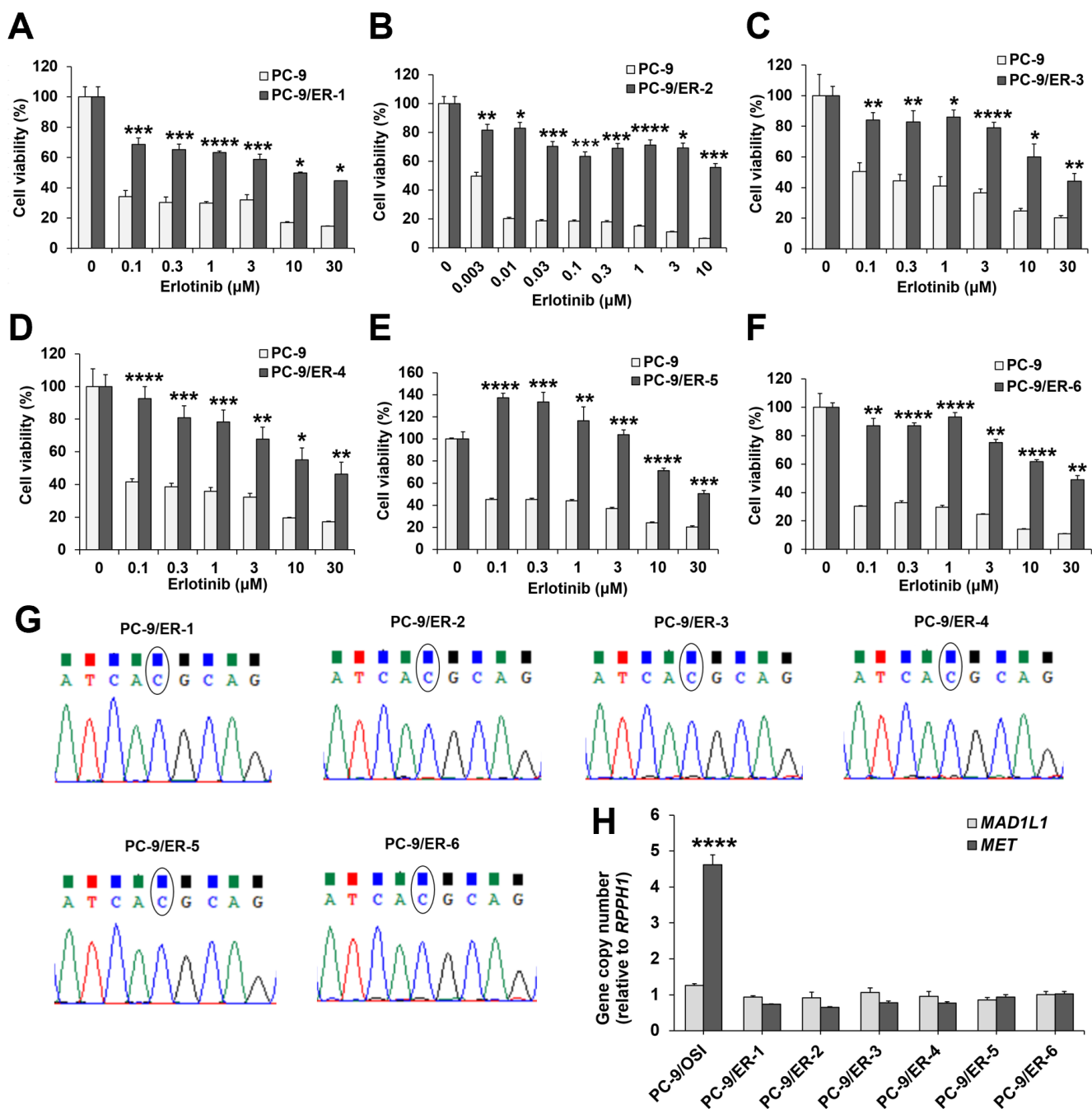


**Figure S2** PD173074 sensitized PC-9/PEM to erlotinib but BLU-554 and nintedanib did not. **A**, Immunoblots of the phosphorylated and total EGFR, the protein binding to anti-pFGFR<sup>Y653/Y654</sup> and total FGFR1 in PC-9 and PC-9/PEM cells treated with PD173074 at the indicated concentration for 96 h. **B**, Immunoblots of phosphorylated- and total PTK2 in PC-9 and PC-9/PEM cells treated with the FGFR1 inhibitor PD173074 at the indicated concentration for 96 h. **C**, Viability of PC-

9/PEM cells with or without PD173074 and pemetrexed at the indicated concentrations for 72 h. Data are means (SD), n = 3. **D**, Viability of PC-9/PEM cells with or without PD173074 and erlotinib at the indicated concentrations for 96 h. Data are means (SD), n = 3. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.0001$  by one-way ANOVA and Tukey's HSD multiple comparisons test. **E**, Immunoblots of the indicated proteins in PC-9 and PC-9/PEM cells with or without 1  $\mu$ M PD173074 and 1  $\mu$ M erlotinib for 72 h. **F**, Immunoblots of cleaved PARP and PARP in PC-9 and PC-9/PEM cells with or without 1  $\mu$ M PD173074 and 1  $\mu$ M erlotinib for 72 h. **G-H**, Viabilities of PC-9 (**G**) and PC-9/PEM (**H**) cells treated with the FGFR4 inhibitor BLU-554 at the indicated concentrations for 96 h. Data are means (SD), n = 3. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.0001$ . Data for treated and untreated cells were compared by one-way ANOVA and Tukey's HSD multiple comparisons test. **I**, viability of PC-9/PEM cells with or without 3  $\mu$ M BLU-554 and 0.01  $\mu$ M gefitinib (Gef), 1  $\mu$ M erlotinib (Erl), 0.001  $\mu$ M afatinib (Afa), or 0.01  $\mu$ M osimertinib (Osi) for 96 h. Data are means (SD), n = 3. \*,  $P < 0.05$ ; NS, not significant by one-way ANOVA and Tukey's HSD multiple comparisons test. **J**, Viability of PC-9/PEM cells with or without BLU-554 and erlotinib at the indicated concentration for 96 h. Data are means (SD), n = 3. NS, not significant by Student's *t*-test. **K**, PC-9/PEM cells treated with the multi-kinase inhibitor nintedanib at the indicated concentrations for 96 h. Data are means (SD), n = 3. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ . Data for treated and untreated cells were compared by one-way ANOVA and Tukey's HSD multiple comparisons test. **L**, Viability of PC-9/PEM cells with or without 0.01  $\mu$ M nintedanib (Ntd) and 0.01  $\mu$ M gefitinib (Gef), 0.01  $\mu$ M erlotinib (Erl), 0.0003  $\mu$ M afatinib (Afa), or 0.03  $\mu$ M osimertinib (Osi) for 96 h. Data are means (SD), n = 3. NS, not significant by one-way ANOVA and Tukey's HSD multiple comparisons test. **M**, Immunoblots of the protein binding to anti-pFGFR<sup>Y653/Y654</sup> in PC-9/PEM clone1 cells transfected with siRNAs against *PTK2*. The siCtrl was used as a negative control.



**Figure S3** Representative pictures and body weights of xenografts. **A**, Xenografted mice with tumors at day 30. Black arrows indicate PC-9 tumors. White arrows indicate PC-9/PEM clone1 tumors. **B**, Body weights up to day 30. Data are means (SD); n = 6. Groups were compared by one-way ANOVA and Tukey's HSD multiple comparisons test.



**Figure S4** Establishment of erlotinib-resistant NSCLC cell lines. **A-F**, Viabilities of PC-9 and PC-9/ER-1 (**A**), PC-9/ER-2 (**B**), PC-9/ER-3 (**C**), PC-9/ER-4 (**D**), PC-9/ER-5 (**E**), and PC-9/ER-6 (**F**) cells treated with erlotinib at the indicated concentrations for 72 h. Data are means (SD), n = 3. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ . Data were compared with those for PC-9 cells by Student's *t*-test. **G**, Direct sequencing chromatogram at the p.T790 (c.2369C) site of EGFR in PC-9/ER-1 – 6. **H**, Copy number quantification of genomic DNA extracted from PC-9, PC-9/ER-1-6, and PC-9/OSI. *MET* or *MAD1L1* copy numbers relative to that for *RPPH1* are shown. *MAD1L1* was the negative control. Data are means (SD), n = 3. \*\*\*\*,  $P < 0.0001$  by Student's *t*-test.

Range 1: 463 to 819 [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
194 bits(494)	2e-55	Compositional matrix adjust.	120/357(34%)	187/357(52%)	34/357(9%)
Query 407	YTMPSTRDYEIQRERIELGRCIGEGQFGDV--HQGIYMSPENP--ALAVAIKTCKNCTSD				462
Sbjct 463	Y +P +E+ R+R+ LG+ +GEG FG V + I + + P VA+K K+ ++				522
Query 463	SVREKFLQEALTMRF-DHPHIVKLIGVITEN-PVWIIMELCTLGELRSFLQVRK-----				515
Sbjct 523	+ E M+ H +I+ L+G T++ P+++I+E + G LR +LQ R+				582
Query 516	-----YSLDLASLILYAYQLSTALAYLESKRFBVHRDIAARNVLVSSNDCVKGDF				565
Sbjct 583	L L+ AYQ++ + YL SK+ +HRD+AARNVLV+ ++ +K+ DF				642
Query 566	GLSRYMEDSTYYK-ASKGKLPIKWMAPESINFRFRFSASDVWVMFGVCMWEILMHGVKPFQ				624
Sbjct 643	GL+R + YYK + G+LP+KWMAPPE++ R +T SDVW FGV +WEI G P+				702
Query 625	GVKNNDVIGRIENGERLPMPPNCPPTLYSLMTKCWAYDPSRRPRFTELKAQLSTILEEEK				684
Sbjct 703	GV ++ ++ G R+ P NC LY +M CW PS+RP F +L L I+				762
Query 685	AQQEERMRM-----ESRRQATVSW--DSGGSDEA-PPKPSRPGYSPRSSEGF				729
Sbjct 763	Q+ + M R +T S DS S E P +P P +P+ ++ G				819

Query: PTK2 (Q05397) Y576/Y577

Sbjct: FGFR1 (P11362) Y653/Y654

**Figure S5** Sequence alignment between PTK2 (Y407 to F729) and FGFR1 (Y463 to L819). Blue indicates serial tyrosine sites of PTK2 and FGFR1.

**Table S1** The sequences for primers used in RT- qPCR.

<b>Gene</b>	<b>Forward primer (5' to 3')</b>	<b>Reverse primer (5' to 3')</b>
<i>IGF-1R</i>	TGGTGGAGAACGACCATATCC	CGATTAAGTGAAGAGGAGTTCTGA
<i>PXN</i>	ACGTCTACAGCTTCCCCAACAA	AGCAGGCGGTTCGAGTTCA
<i>ITGβ1</i>	CATCTGCGAGTGTGGTGTCT	AAGGCTCTGCACTGAACACA
<i>Gab1</i>	ATCAGAAACGCCAGCGAAGA	TCAGATACCACAAAGCACCA
<i>Gab2</i>	ACAGTACCTACGACCTCCCC	CTGGGCGTCTTGAAGGTGTA
<i>Jak1</i>	AGACTTGTGAATACGTAAAAGAAGGA	AAAGCTTGTCCGATTGGATG
<i>ErBb2</i>	TGTGACTGCCTGTCCCTACAA	CCAGACCATAGCACACTCGG
<i>PDGFRβ</i>	GCACCGAAACAAACACACCTT	ATGTAACCACCGTCGCTCTC
<i>MET</i>	CCATCCAGTGTCTCCAGAAGTG	TTCCCAGTGATAACCAGTGTGTAG
<i>MER</i>	ACAGGTTCTGGGACGTCCATC	CCGGAATAGCGGGTAAGGC
<i>PIK3CA</i>	AATGCTTGGGGTGAAGGGAC	GGGGTGCAAACAATGCATGAC
<i>AXL</i>	TACCGCCAGGGACGTATCGC	CCAGCACCGCGACATCAAGG
<i>GAS6</i>	TGGCGCGGAATCTGGTCATC	GAAGCACTGCATCCTCGTGTTT
<i>PDPK1</i>	CAGAGGTCAGGCAGCAACATAGAG	ACGTCCTGTTAGGCGTGTGG
<i>EPHA2</i>	CCGGCTACACTGCCATCGAG	GCCCAGCATCCCTGGTCATC
<i>TYRO3</i>	AACATCTTGGGCCAGCTGTCTG	GATTTGGTCAGTCCGGGCTTC
<i>PTEN</i>	GACCCACACGACGGGAAGAC	GCCTCTGGATTGACGGCTCC
<i>TWIST1</i>	CATGTCCGCGTCCCACTAG	TGTCCATTTTCTCCTTCTCTGG
<i>PTK2</i>	GCCTTAACAATGCGTCAGTTTGACC	AAATGACCTCAGCTCTCCAAGTGTG
<i>FGFR1</i>	GCATCATAATGGACTCTGTGGTG	GTGGTTGATGCTGCCGTAATC
<i>FGFR2</i>	CGCTGGTGAGGATAACAACACG	TTCCGCCATGACCACTTGCC
<i>FGFR3</i>	TACCGTGCTCAAGGTGTCCC	TTGCAGGTGTGAAGGAGTAGTC
<i>FGFR4</i>	GGGGAGAACCGCATTGGAGG	ACACGTTCCGCAGGTACAGG
<i>GAPDH</i>	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGA