Supporting Information

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SI Materials and Methods

Direct Sequencing of *NRAS*. Genomic DNA was extracted from patient samples (>70% tumor cells) and cell lines using standard procedures. NRAS exons 2 and 3 were amplified from genomic DNA and were sequenced directly. The sequences of primers for NRAS were as follows:

Human NRAS exon2:
5′-ACCAAATGGAAGGTCACACTAGGGTTT-3′ (forward primer),
5′-ACAGGATCAGGTCAGCGGGC-3′ (reverse primer)
Human NRAS exon3:
5′-TGAGGGACAAACCAGATAGGCAGA-3′ (forward primer)
5′-CCCTAGTGTGGTAACCTCATTTCCCCA-3′ (reverse primer)

FISH. Cells were grown in RPMI 1640 with 10% FBS to ∼70% confluence. One hour before harvest, colcemid (Invitrogen) was added to each flask containing 10 mL growth medium. Metaphase FISH slides were prepared as previously described (1). The MET/CEP7 probe set was from Cytocell. The probe targeting *MET* was labeled in red, and the chromosome 7 centromere probe (CEP7) was labeled in green. The EGFR/CEP7 probe set was from Abbott Molecular. Probe targeting *EGFR* was labeled in red, and the CEP7 probe was labeled in green. Dual-color FISH was performed according to the protocol from Vysis/Abbott Molecular with a few modifications. Nuclei were counterstained with DAPI (blue). FISH signal was captured using a fluorescence microscope (Zeiss) coupled with an ISIS FISH Imaging System (MetaSystems) following the manufacturer's instructions. Metaphases showing optimum hybridization signals were scored. Five representative metaphases from each sample were captured.

1. Bean J, et al. (2007) MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 104:20932–20937.

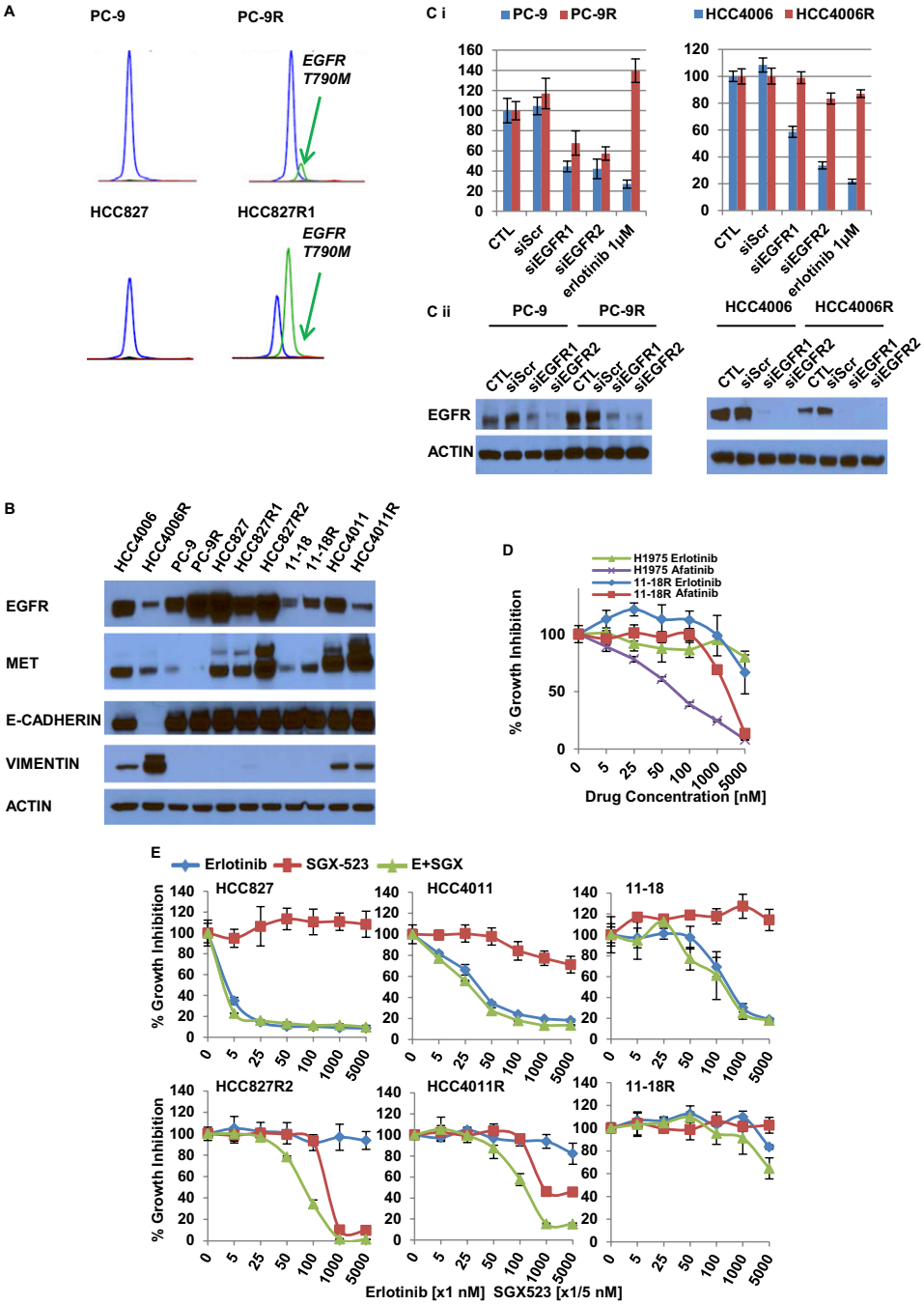

Fig. S1. Characterization of *EGFR*-mutant cell-line models of acquired resistance. (*A*) SNaPshot assay reveals that PC-9R and HCC827R1 cells have acquired an *EGFR* T790M mutation. (*B*) Immunoblotting studies with the indicated antibodies show that MET is overexpressed in HCC827R2, 11-18R, and HCC4011R cells compared with their respective parental cells. HCC4006R cells display features of epithelial–mesenchymal transition, i.e., increased expression of vimentin and loss of E-cadherin. (*C*) The effect of siRNA-mediated knockdown of EGFR in PC-9/PC-9R cells and HCC4006/HCC4006R cells. (*D*) Cell growth-inhibition assays show the relative sensitivity of 11-18R and H1975 cells to erlotinib and to the irreversible EGFR TKI, afatinib. (*E*) Cell growth-inhibition assays using various EGFR-mutant cell lines and treatment with erlotinib, the MET inhibitor SGX-523, or the two combined. Data shown in *C*, *D*, and *E* are mean ± SD of three independent experiments performed in hextuplicate.

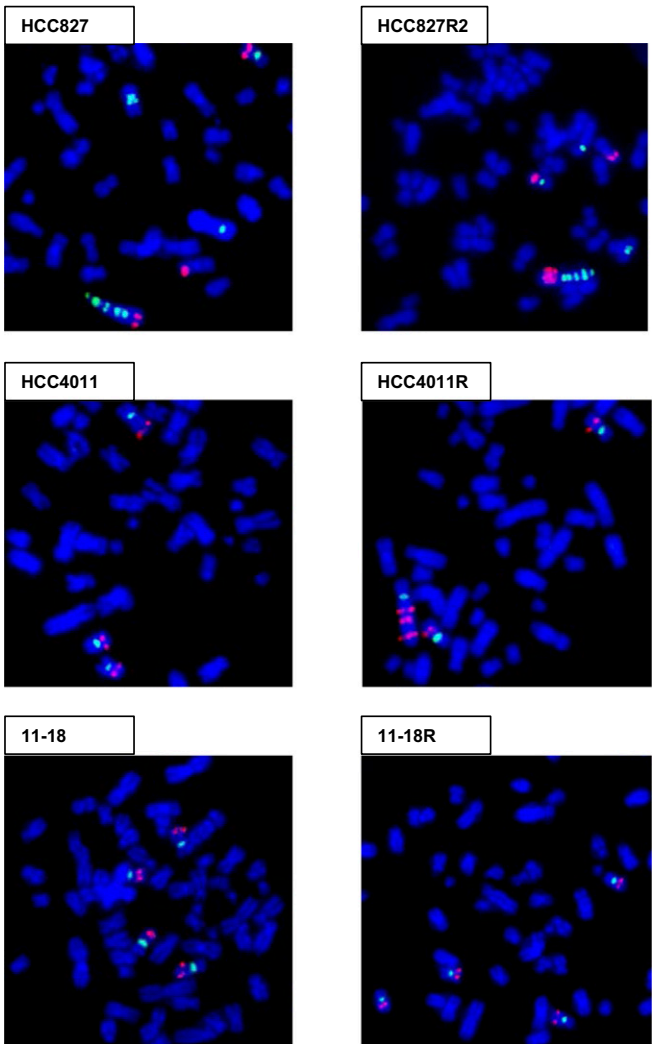

Fig. S2. *MET* FISH analysis in *EGFR*-mutated cell lines. *MET* is labeled in red; CEP7 is labeled in green. Compared with the parental cells, high-level amplification of MET was noticed in HC827R2 cells, and a few additional copies of MET (amplification) were detected in HCC4011R cells. No significant difference was observed between 11-18 parental and 11-18R cells.

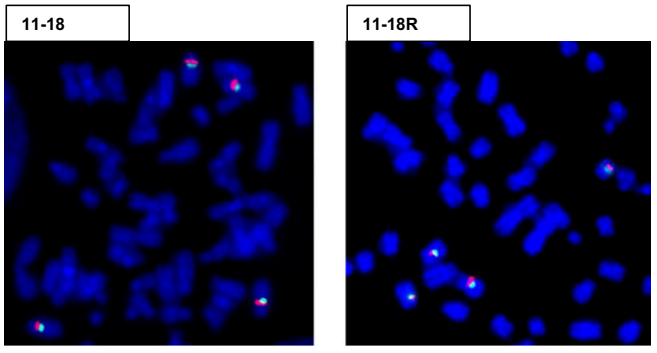

Fig. S3. *EGFR* FISH analysis in 11-18 and 11-18R cell lines. *EGFR* is labeled in red; CEP7 is labeled in green. No significant difference was observed between 11-18 parental and 11-18R cells.

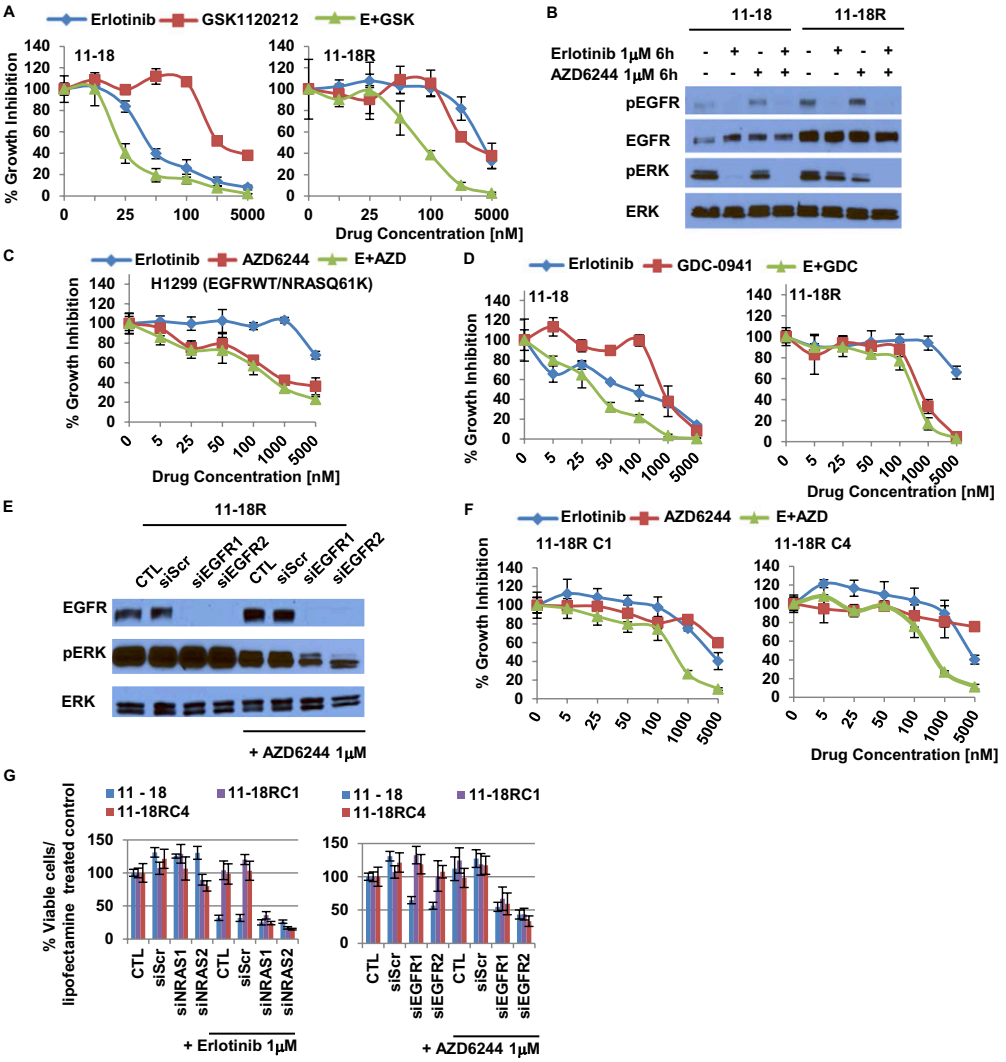

Fig. S4. Additional studies on the functional role of *NRAS* Q61K in 11-18R cells. (*A*) Cell growth-inhibition assays show that 11-18R cells are sensitive to combined inhibition with erlotinib and the MEK inhibitor GSK1120212. (*B*) Immunoblotting studies with the indicated antibodies show the effect of erlotinib, AZD6244, or the combination on EGFR signaling in 11-8/11-18R cells. (*C*) H1299 cells harboring wild-type *EGFR* and *NRAS* Q61K display sensitivity to AZD6244; erlotinib did not have any significant additive effect. (*D*) Cell growth-inhibition assays show that addition of the selective PI3K inhibitor GDC-0941 to erlotinib did not enhance sensitivity of 11-18R cells. (*E*) Immunoblotting studies using the indicated antibodies show that siRNA-mediated knockdown of EGFR combined with AZD6244 inhibited ERK activation in 11-18R cells. Scr, Scramble. Two different siRNAs against EGFR were used. (*F* and *G*) The effect of EGFR, NRAS, or MEK inhibition with small molecules (*F*) and/or siRNAs (*G*) on cell growth in the single-cell clones 11-18RC1 or C4. Data shown in *A*, *C*, *D*, *F*, and *G* are mean ± SD of three independent experiments performed in hextuplicate. Scr, Scramble. Two different siRNAs against NRAS or EGFR were used.

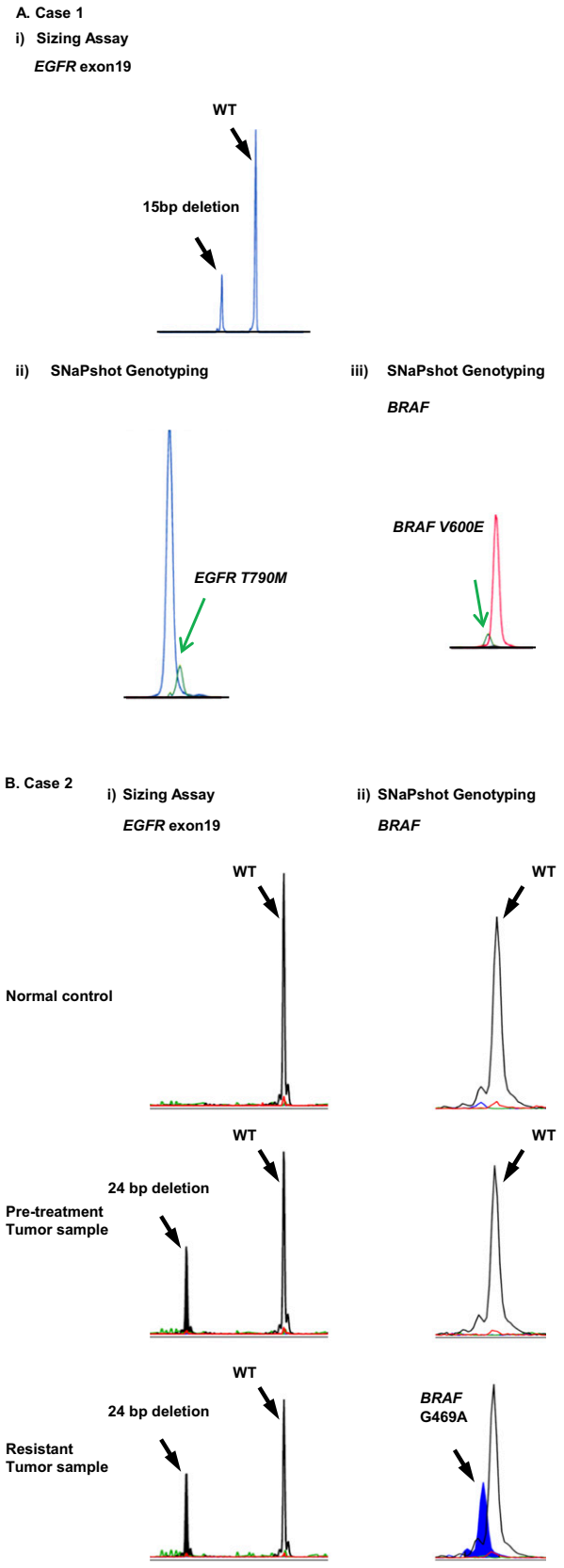

Fig. S5. Mutation status of *EGFR* and *BRAF* in patients with acquired resistance to erlotinib. (*A*) Case1. (*i*) The peak on the right indicates wild-type *EGFR* at codon exon 19. The peak on the left shows mutant *EGFR* (15-bp deletion at codon 19). (*ii*) The green peak indicates the existence of *EGFR* T790M in the tumors. (*iii*) The green peak indicates a *BRAF* V600E mutation. (*B*) Case 2. (*i*) The peak on the right indicates wild-type *EGFR* at codon exon 19. The peak on the left shows mutant *EGFR* (24-bp deletion at codon 19). (*ii*) The peak on the right indicates wild-type *BRAF*. The blue peak on the left shows mutant BRAF G469A.

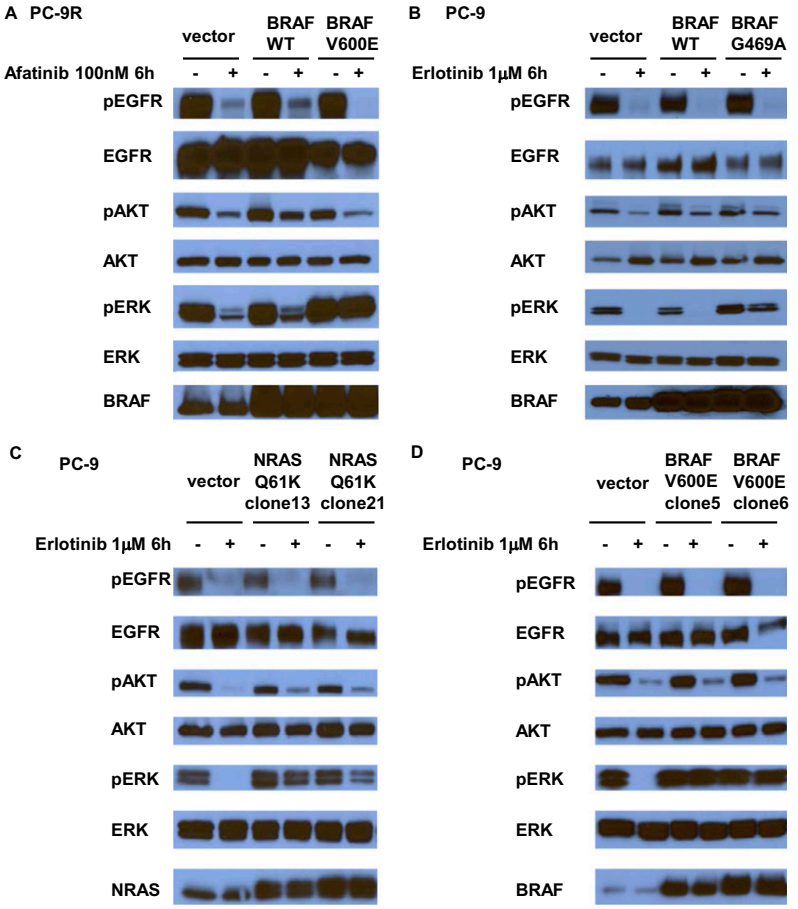

Fig. S6. Ectopic expression of *NRAS* Q61K, *BRAF* V600E, or *BRAF* G469A in *EGFR*-mutant cells abrogates sensitivity to EGFR tyrosine kinase inhibitors. (*A*) PC-9R cells were transiently transfected with expression plasmids encoding wild-type *BRAF* or *BRAF* V600E and were cultured in the absence or presence of afatinib for 6 h. Corresponding cell lysates were subjected to immunoblotting with the indicated antibodies. (*B*) PC-9 cells were transiently transfected with expression plasmids encoding wild-type *BRAF* or *BRAF* G469A and were cultured in the absence or presence of erlotinib for 6 h. Corresponding cell lysates were subjected to immunoblotting with the indicated antibodies. (*C* and *D*) PC-9 cells were stably transfected with control plasmids or expression plasmids encoding *NRAS* Q61K (*C*) or *BRAF* V600E (*D*) and were cultured in the absence or presence of erlotinib for 6 h. Corresponding cell lysates were subjected to immunoblotting with the indicated antibodies.

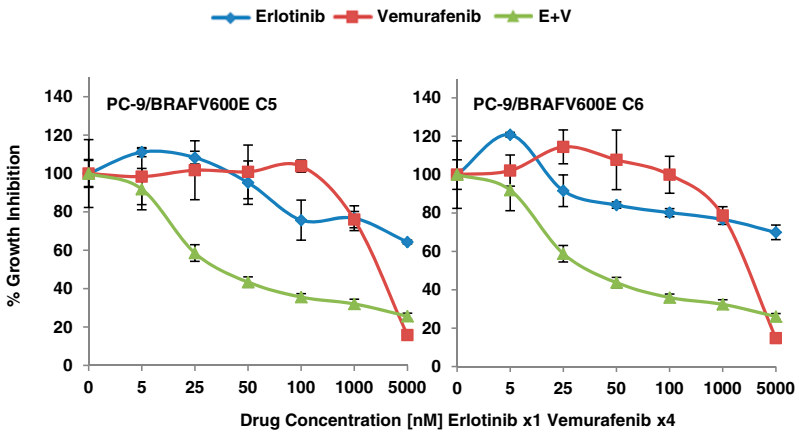

Fig. S7. BRAF inhibition restores the sensitivity of PC-9/BRAF V600E stable clones to erlotinib. The combination of erlotinib and vemurafenib leads to greater cell growth-inhibition of PC-9 cells stably expressing *BRAF* V600E (PC-9/*BRAF* V600E cells) than seen with either drug alone. Data shown are mean ± SD of three independent experiments performed in hextuplicate.

Table S1. SNaPshot results in 11-18R cells

Locus	WT nt	MT nt	Amino acid mutation	Result
AKT1.49_R	G	A	E17K	WT
BRAF1397_F	G	T	G466V	WT
BRAF1406_F	G	C	G469A	WT
BRAF1789_F	C	G	L597V	WT
BRAF1799_F	T	A	V600E	WT
EGFR2155_F	G	A	G719S	WT
EGFR2155_F	G	T	G719C	WT
EGFR2156_F	G	C	G719A	WT
EGFR2369_R	C	T	T790M	WT
EGFR2573_F	T	G	L858R	MT
EGFR2582_R	T	A	L861Q	WT
KRAS_183R	A	C	Q61H	WT
KRAS_37F	G	A	G13S	WT
KRAS_37F	G	C	G13R	WT
KRAS181_F	C	A	Q61K	WT
KRAS182_F	A	T	Q61L	WT
KRAS182_F	A	G	Q61R	WT
KRAS183_R	A	T	Q61H	WT
KRAS34_R	G	C	G12R	WT
KRAS34_R	G	T	G12C	WT
KRAS34_R	G	A	G12S	WT
KRAS35_F	G	A	G12D	WT
KRAS35_F	G	T	G12V	WT
KRAS35_F	G	C	G12A	WT
KRAS37_F	G	T	G13C	WT
KRAS38_F	G	A	G13D	WT
KRAS38_F	G	C	G13A	WT
MEK1.167_F	A	C	Q56P	WT
MEK1.171_R	G	T	K57N	WT
MEK1.199_R	G	A	D67N	WT
NRAS181_F	C	A	Q61K	MT
NRAS182_F	A	T	Q61L	WT
NRAS182_F	A	G	Q61R	WT
PI3K1624_R	G	A	E542K	WT
PI3K3140_R	A	G	H1047R	WT
PIK3CA1633_F	G	C	E545Q	WT
PIK3CA1633_F	G	A	E545K	WT
PTEN697_R	C	T	R233*	WT

MT, mutant; nt, nucleotide.
*Truncating mutation.

Table S2. Genotypes of 10 different single-cell clones derived from 11-18R cells

Clone	*EGFR* L858R	*EGFR* T790M	*NRAS* Q61K
11-18R C1	+	–	+
11-18R C2	+	–	+
11-18R C3	+	–	+
11-18R C4	+	–	+
11-18R C5	+	–	+
11-18R C6	+	–	+
11-18R C7	+	–	+
11-18R C8	+	–	+
11-18R C9	+	–	+
11-18R C10	+	–	+

C, clone, +, detected; –, not detected.