Supplemental Materials and Methods

Mass Spectrometry. The human recombinant $EGFR^{L858R/T790M}$ protein was obtained from BPS Bioscience (San Diego, CA). Intact $EGFR^{L858R/T790M}$ protein was incubated at room temperature for 1 hour with 10-fold excess of compounds at 37°C. At the end of the incubation, 5 µL aliquots of the samples were diluted with 15 µL of 0.2% TFA and then purified/concentrated with C4 ZipTips® and directly spotted onto a MALDI target plate using Sinapinic acid as the desorption matrix (10 mg/mL in 0.1%TFA in acetonitrile:water 50:50 v/v). Finally, each spot was analyzed on an ABSciex 4800 MALDI TOF-TOF mass spectrometer fitted with a HM2 detector (CovalX Saugus, MA). The centroid mass of intact $EGFR^{L858R/T790M}$ protein with or without compound was determined to assess % mass modification of $EGFR^{L858R/T790M}$ protein.

Supplemental Figure Legends

Supplemental Figure 1. Signaling inhibition in EGFR wild-type (A431) and mutant EGFR NSCLC cells (H1975 and HCC827). Representative immunoblots are shown. Tubulin levels are shown as loading controls.

Supplemental Figure 2. Prolonged duration of action on $EGFR^{L858R/T790M}$ (A) and $EGFR^{DelE746\text{-}A750}$ (B) proteins. Representative immunoblots are shown. H1975 and HCC827 cells were treated with 500 or 1000 nM, respectively, of compound 3 for 1 hr and then extensively washed to remove any remaining compound. Cells were re-fed with complete (10% FBS) medium for the indicated time points. Cell lysates were assessed for pEGFR, pAKT, pERK and target occupancy (H1975 only). Tubulin levels are shown as loading controls.

Supplemental Figure 3. Protein degradation ($t_{1/2}$) of $EGFR^{L858R/T790M}$ (A) and $EGFR^{DelE746\text{-}A750}$ (B) in H1975 and HCC827 cells, respectively. Cells were starved overnight and then treated with cycloheximide (10 µg/mL) for the indicated times. Total $EGFR^{L8585R/T790M}$, $EGFR^{DelE746\text{-}A750}$ and Myc protein degradation was determined by immunoblot analysis. All signals were normalized to tubulin expression. Myc expression was used as a control and has a known $t_{1/2} < 2$ hr. % Inhibition was normalized to % DMSO control. $n \geq 3$; Ave ± STD.

Supplemental Figure 4. Prolonged duration of action on $EGFR^{DelE746\text{-}A750}$ protein by erlotinib. HCC827 cells were treated with approximately 10-fold EC_{50} (100 nM) for 1 hr. After treatment cells were extensively washed to remove any remaining compound. Cells were re-fed with complete (10% FBS)

medium for the indicated time points. Cell lysates were assessed for pEGFR inhibition. n = 3; Ave ± STD.

Supplemental Figure 5. Time-dependent inhibition in EGFR mutant H1975 (A) and HCC827 (B) cells. Representative immunoblots are shown. H1975 and HCC827 cells were treated at 10-fold EC_{50} of compound 3, 500 and 1000 nM, respectively, for the indicated times. Lysates were evaluated for pEGFR, pAKT and pERK. For H1975 lysates, target occupancy was also assessed. Tubulin levels are shown as loading controls.